Reproductive investment and strategies of *Gobiomorphus cotidianus*

A thesis submitted in partial fulfilment
of the requirements for the degree
of
Masters of Science (Research)
in Biological Sciences
at
The University of Waikato
by

Kate Louise Pepper

The University of Waikato
2015

THE UNIVERSITY OF
WAIKATO
Te Whare Wānanga o Waikato

Abstract

The New Zealand common bully *(Gobiomorphus cotidianus)* is a member of the family *Eleotridae* which comprises around 35 genera and some 150 species worldwide. *G. cotidianus* are abundant throughout New Zealand and thrive in streams, large rivers and lakes, with broad tolerances for temperature, pH, flow and lake trophic status. It is known that male bullies clean and guard a nest site and attract one or possibly more females to spawn within the nest which is then guarded until the eggs hatch. However, due to intraspecific competition, some males miss out. Male alternative reproductive tactics (MARTs) are commonly used to achieve a successful reproductive outcome when the usual reproductive strategy becomes energetically inefficient for the individual. MART's are common within the closely related family *Gobiidae* but have not yet been observed in the *Eleotridae.*

Differential investments in gonadal mass versus parental care are known in many animals from fish to humans. Greater parental investment typically correlates with smaller gonad size. I investigated the relative investment made by male common bully in gonads versus seminal vesicles on the assumption that if non-guarding (parasitic) males occur, they would invest significantly less effort in seminal vesicles (SVs) compared with "bourgeois" (nest guarding) males. I examined these traits monthly to determine possible seasonal changes in reproductive strategies. We found that male investment in SVs does vary significantly between individuals and that the greatest investment in this structure in males coincides with the peak summer breeding season. A small proportion of males (usually larger individuals) invest heavily in SVs but it is currently unknown whether these, and only these, defend nest sites and whether the remainder rely on MART strategies for reproductive success. Seminal vesicle size was positively correlated with fish size whereas testis size declined with increasing size of males.

Sampling of common bully from Lake Karapiro began in December 2013 and finished in January 2015 providing fourteen months of data to

predominantly investigate male reproductive investment using calculations of gonadosomatic index (GSI) and seminal vesicle somatic index (SVSI). I found that although investment in both gonads and seminal vesicles increase in preparation for the breeding season, the increase in SVSI is considerably greater than the comparatively slight increase in GSI. I also examined female GSI and found that it followed similar trends to that of the SVSI with sharp increases occurring throughout late winter and spring.

Calculations of somatic condition indices were made for both males and females throughout the 14 month sampling period. This identified similar condition trends for both sexes with peaks throughout summer suggesting a slight increase in somatic condition post-breeding season. Male condition was slightly higher and more varied during this summer period.

A behavioural experiment was carried out to investigate male nest preference and guarding behaviour. Obvious nest guarding was displayed in 14 of the 40 nest arenas and 11 of the guarded nests were large pots compared with only 3 guarded small pots. GSI and SVSI data from the males within the experiment suggest an increased investment in seminal vesicles by males displaying nest guarding behaviour. However, the sample size for both of these elements was too small to provide statistically significant results.

Acknowledgements

Firstly I would like to thank my supervisor Nick Ling whose enthusiasm for the project provided me with the motivation and independence to make it through my masters. Thank you for the countless hours spent cutting up buckets and helping me nut out all of my uncooperative fish problems. Your patience in teaching me the tricks on the best ways of dissecting gonads out of miniscule fish paid off. By always keeping my eyes on the target rather than the tool and making the cuts between breathing, not only did I keep all my fingers, but if a career in science doesn't pan out, I might make a good sniper one day.

Thank you to my 'Advanced Technical Officer' Warrick Powrie for keeping me amused with your singing on our road trips to and from the lake every few weeks rain or shine, and for not carrying out your threat to push me out of the boat as a practical man over board training session. Thanks for always answering your phone regardless of the day or time and when all else failed, for supplying me with V and chocolate to keep me quiet. To our other department technicians Lee and Dudley and research fellow Grant, your help both in the field and in the lab always made university life that much easier and was much appreciated, especially Lee's baking and Grant's "motivational advice".

I could not have made it through the last two crazy years without my fellow masters students. Alicia, Jeremy, Jordan, Nikki, Ashley, and Laura, your support, advice and positivity picked me up more times than I can count. The food parcels, energy drinks, alcohol and baking deliveries were brilliant and your arguably unhealthy love of science made me feel at home in good company at all times.

To Michael, thank you for nodding and agreeing every time I needed to vent, even when you had no idea what I was talking about. For putting up with me when I was too tired to remember even my own name and forgiving me for taking over your computer, room and weekends with SCIENCE! I will be forever grateful for your tech support, calming words

and ability to put a smile on my face even when all I wanted to do was punch something, especially in the final weeks of my write up. Your willingness to spend your new year's day rowing across the middle of a lake in a dingy dodging jet skiers and wake boarders to collect minnow traps was above and beyond.

My heartfelt gratitude to my amazing parents who have not only put up with my grumpy sleep deprived state for the last few months but undoubtedly my whole life. Your love and support throughout this whole journey means more than I can say. Mum for telling me it will all be worth it in the end, even when I didn't want to hear it and dad for sparking and fostering my love for fish and all things freshwater I could never have dreamt of doing this without you guys.

I owe an apology to my friends and family outside of university for neglecting you for so long and forgetting to reply to your messages, emails and calls, especially Mitch. It has been a long year, looking back I have missed you all so much more than I ever expected, and can't wait to catch up with you all for numerous drinks once I have slept for a few weeks. If I have missed anyone out I am sorry! It is not on purpose, just put it down to a severe case of "thesis brain".

Finally I owe an apology to my big brother and role model Brad, I guess this means you have to do your PhD now.

"The most beautiful experience we can have is the mysterious - the fundamental emotion which stands at the cradle of true art and true science."
— Albert Einstein

Table of Contents

List of Figures

Chapter One - General Introduction

This thesis examines the reproductive biology of the common bully *Gobiomorphus cotidianus*, a small endemic freshwater fish species found throughout the freshwater ecosystems of New Zealand. Differential investments in the mass of the male reproductive structures were investigated on a monthly basis in relation to parental care, seasonality and alternative reproductive strategies. We examined the energetic investment made by the male common bully in gonad versus seminal vesicle size on the assumption that the ratio of investment between the two reproductive organs would shift in relation to the reproductive strategy of the individual male. This thesis also investigates some of the factors involved in mate choice and nest site selection including size, coloration and behaviour.

1.1 *Eleotridae*

The family *Eleotridae* (sleepers) include around 35 genera and 150 species worldwide, distributed most commonly throughout the tropical and temperate Indo-Pacific regions (Figure 1.1). Most eleotrids are small benthic dwelling fish ranging in size between 30 mm and 100 mm total length with a small number of larger bodied free swimming species, the largest being the marble goby *Oxyeleotris marmorata*, growing to 66 cm total length (Berra, 2001). Whilst the majority of the species within the family reside primarily in freshwater, the remainder of the species reside in brackish ecosystems such as mangrove forests with only a few eleotrids residing solely in the marine ecosystem (Berra, 2001). Many of the freshwater species display a diadromus migratory life phase where the larvae migrate or are washed out to sea before returning to freshwater in juvenile form; this form of diadromy is known as amphidromy.

The family *Eleotridae* are split into two sub-orders *Butinae* and *Eleotrinae,* and within the *Eleotrinae* is the genus *Gobiomorphus* which includes nine known species with a distribution limited to Australia and New Zealand. Two of the nine species are endemic to Australia, Cox's gudgeon,

Gobiomorphus coxii and striped gudgeon, *Gobiomorphus australis,* both of which are freshwater species (Gomon, 2011a, Gomon, 2011b).

The seven remaining species within the *Gobiomorphus* genus are all endemic to New Zealand and are more commonly known as bullies. Bullies are the second largest family group making up the New Zealand endemic freshwater fish fauna. The seven species include the Cran's bully *(G. basali*s), giant bully *(G. gobioides),* upland bully *(G. breviceps),* common bully *(G. cotidianus)*, bluegill bully *(G. hubbsi)* Tarnedale bully *(G. alpinus)* and the redfin bully *(G. huttoni)* (McDowall, 1990).

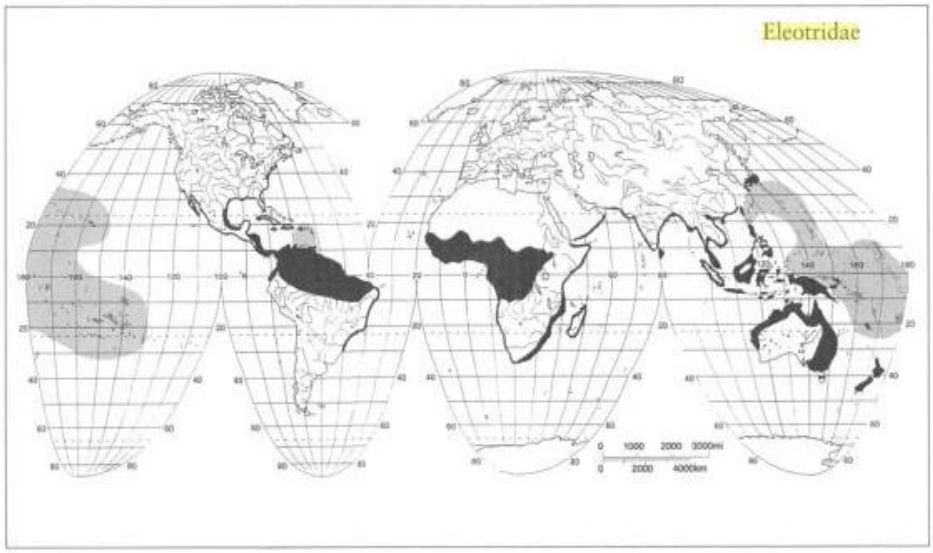

Figure 1.1: The worldwide distribution of *Eleotridae*- shaded black (Berra, 2001)

1.2 The Biology of New Zealand's Endemic *Gobiomorphus* Species

The seven New Zealand bully species have adapted to inhabit a wide variety of aquatic ecosystem types including riverine, stream, estuarine, lakes, ponds and tarns. There appears to be a combination of specialist habitat species and generalist habitat species (McDowall, 1990). The Tarndale bully is thought to be an ecotype of the common bully (Smith et al, 2003 & McDowall & Stevens, 2007) and is only found in five high alpine tarns in the South Island (McDowall, 1994), whereas the giant bully is typically observed in estuarine ecosystems throughout New Zealand with very little penetration inland up streams or rivers. The redfin bully is often found near the coast in swifter currents, similar to the bluegill bully whose

finer body shape is well adjusted to inhabit fast flowing waters such as streams and rivers. The generalist habitat species include the upland bully, commonly found in lakes, streams and rivers throughout the South Island and lower North Island and the Cran's bully found in similar ecosystems throughout the North Island. The common bully is the most widespread of all seven species (Figure 1.2) with the ability to inhabit a wide variety of ecosystem types due to its increased tolerance of environmental factors such as temperature, dissolved oxygen and turbidity (McDowall, 1990).

The common bully can be found in both large and small lakes, rivers and streams (see Figure 1.3 for distance and altitude penetration) with some populations being introduced rather than naturally occurring. Common bully have been noted as being actively introduced as an important prey species for the increasing salmonid and eel fisheries in New Zealand, being intentionally stocked in freshwater systems as a food source for trout (NZ Waterways restoration, 1995).

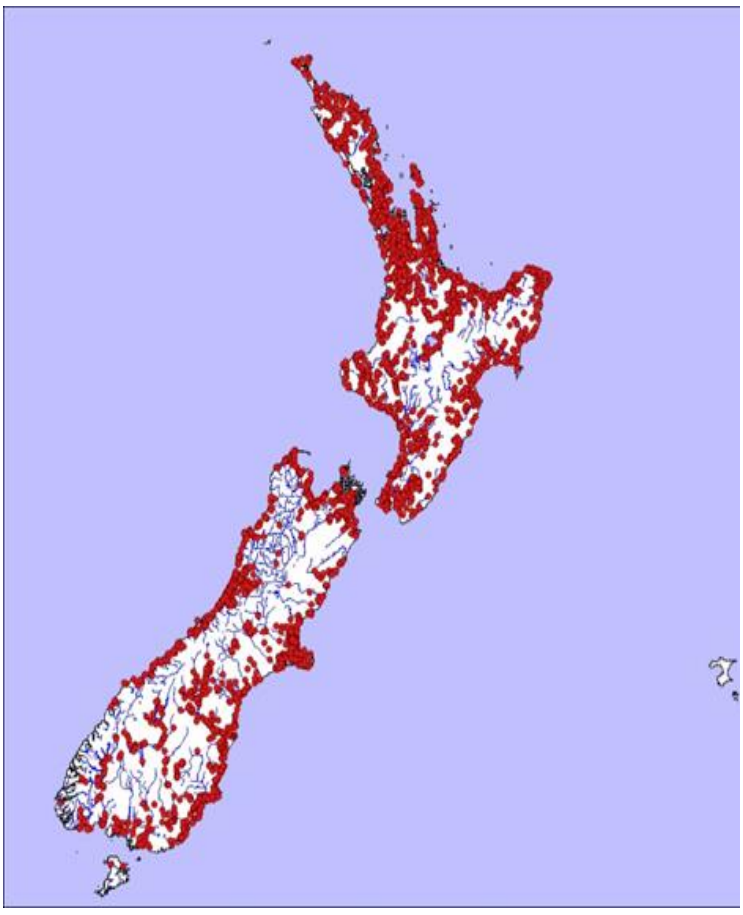

Figure 1.2: Known distribution of the common bully throughout New Zealand (NIWA Freshwater Fish Database)

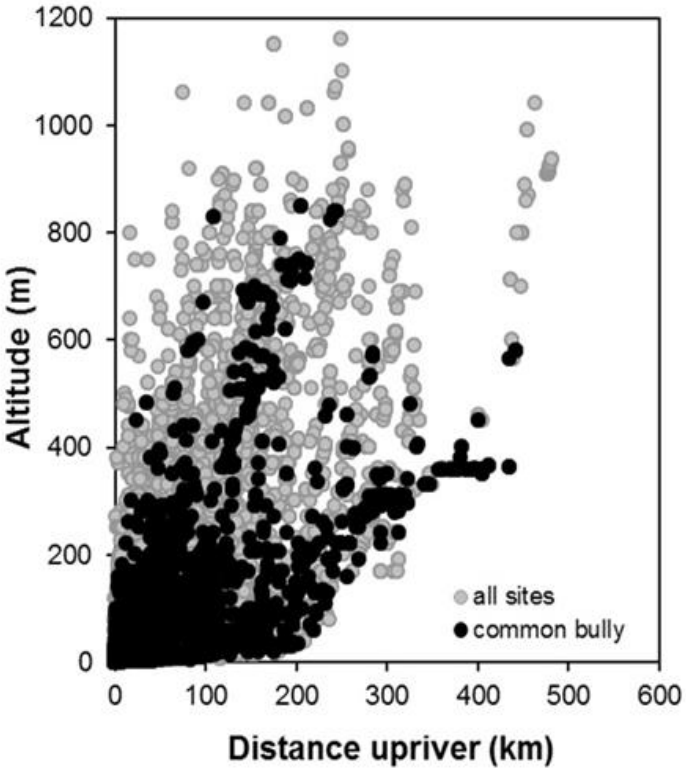

Figure 1.3: Known penetration of common bully distance inland and altitude range (NIWA Freshwater Fish Database)

There has been a lack of research into the general reproductive biology of the common bully across multiple habitats, with one of the more complete biological studies being carried out within Lake Waahi, by Stephens (1978). Although the research is a well-rounded account of the biology of the species, it is still limited due to the research being confined to the interactions of a single population within one study site. It is also limited by the lack of long term historical data sets that would allow for trends to be more accurately observed over a period of years and rule out data limited by specific climatic and environmental factors occurring with the years of field experimentation and research.

Stephens' (1978) research focused on the population distribution patterns in relation to fish age classes and what factors governed the movement and distribution of fish within the lake environment. Other aspects of the research included fish growth, condition, mortality and spawning in relation to season as well as parasitism and larval development. The main findings

of this research suggest that spawning peaks occurred in August, November and February with the sex ratio of males to females in the catch decreasing during peak spawning, with males dropping to 10% during that time (Stephens, 1982). Juvenile growth was fastest during summer whilst adult growth was fastest during winter, and mortality rates increased with age and peaked during summer. Observations of distribution suggest that bullies gathered in the littoral zone in summer with adults occupying shallow waters at night and being replaced by juveniles during the day, whereas all age classes remained in the deeper sections of the lake throughout the winter period.

The reproductive biology of the closely related *Gobiomorphus breviceps* was examined by Hamilton et al. (1997) in an attempt to identify the factors that influence the wide variability in male reproductive success within a population. However, no definitive correlations between male size, nest site characteristics or parasite load could be attributed to these fluctuations. The authors suggested that this was most likely due to highly dynamic stream ecosystems in which they were sampling, with floods and droughts occurring in both study sites possibly affecting the physical structure of the stream and associated biological responses by the fish to the fluctuating ecosystem events. Male territory, nest habitat and female choice were suggested as being the most likely contributing factors in the variable male reproductive success rate of *G. breviceps*.

The common bully is one of 11 New Zealand freshwater fish species classified as not threatened, meaning they are resident native taxa with large, stable populations (Goodman et al. 2013). However, irrespective of their population size and vast distribution, research into the reproductive biology of the New Zealand *Gobiomorphus* is still distinctly lacking across all seven species.

The findings from this thesis will hopefully aid in the development of knowledge surrounding reproductive biology of the genus *Gobiomorphus* and build upon previous findings to include previously over-looked aspects of their reproductive biology. Research is yet to be undertaken on the

mating strategies of Eleotrids, even though there is significant literature on the distribution and occurrence of male alternative reproductive techniques (MART's) in the closely related gobies.

1.3 Migratory Ecotypes

The New Zealand endemic freshwater fish fauna are relatively unique due to the high percentage of species requiring a form of diadromy to carry out their life cycle. Diadromy is a migratory behaviour displayed by numerous native freshwater species in which the fish spend a portion of their life in the ocean in order to complete their life cycle. There are three different forms of diadromy; New Zealand *Gobiomorphus* species that are diadromus display amphidromy. Amphidromus adults reside entirely in freshwater, after spawning the hatched eggs get swept out to sea in larval form where they develop into juveniles. Once they have grown large enough to have the physical ability to swim back upstream against the current they return to freshwater to complete their life cycle (Figure 1.4). Amphidromus fish are either obligately amphidromus of facultatively amphidromus (McDowall, 1993). Obligates appear to require amphidromus behaviour in order to complete their life cycle, whereas facultative species can complete their life cycle without amphidromy, either prevented from entering marine waters due to physical barriers or by choice. However they will often display amphidromus behaviour if the environmental conditions favour migration (Northcote & Ward, 1985; McDowall, 2001).

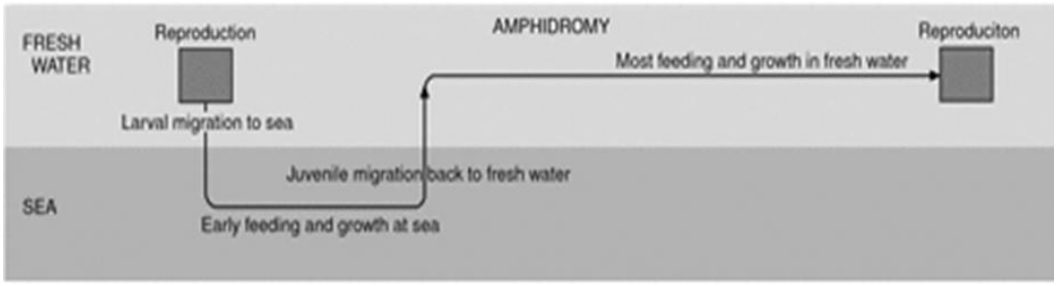

Figure 1.4: Depiction of the basic migratory life cycle of an amphidromus freshwater fish. (McDowall, 1997)

Catadromy is a migratory behaviour for the purpose of reproduction, in which the juvenile to adult phase of development occurs in freshwater

followed by sexually mature adults migrating to sea in order to reproduce. Juvenile fish then migrate back into the fresh water to continue development, but post-reproductive adults do not return. Anadromy, although also for the ultimate purpose of reproduction, is the opposite of catadromy, in which the majority of growth and development occurs at sea and the sexually mature adults migrate into fresh water to reproduce. Hatched larvae are then washed or migrate back downstream into the sea to carry out their life cycle.

Common bullies are classed as being a facultative species with the ability to display both diadromus and non-diadromus tendencies depending on the habitat that they are residing in (van den Heuvel et al, 2007). Riverine populations that have access to the ocean will often display amphidromy if their adult home range is in the lower reaches of the river system (Sutherland & Closs, 2001) resulting in larval drift out to sea, or if current habitat conditions are unfavourable (Closs et al. 2003). If riverine habitats provide a suitable rearing environment for juveniles, then a migratory strategy may be abandoned forming resident non-diadromus populations (Closs et al. 2003; Bleackley et al. 2009; Landman et al, 2010). Lacustrine populations can be land-locked either naturally or anthropogenically by barriers such as irrigation dams and hydro power stations. These barriers prevent larval migration, forcing the population to carry out their complete life cycle in freshwater.

Non-diadromus populations that do not have the ability to migrate or interbreed with other populations can become genetically distinct over time as a result of isolation. A local example of this was a genetic study done with *G. breviceps*, involving 89 fish from 19 catchments throughout the country. Analysis of the mitochondrial DNA identified two genetically distinct geographical clades including the northern clade (occupying the North Island and north western South Island) and the south eastern clade (Smith et al, 2005).This identification of genetic divergence occurred in the absence of phenotypic divergence although obvious phenotypic and morphological differences are common within the genus.

Morphological changes can be a common occurrence between riverine and lake ecotypes of *G. cotidianus* with phenotypic differences noted. These differences can be instrumental in identification with the most common and easily identifiable differences being the number or lack of open pores on the head. A recent study by Vanderpham (2012) investigated the variation in the lateral line mechanosensory systems when comparing the fluvial habitat specialist species *G. huttoni* with the habitat generalist, *G. cotidianus*. This research showed the variation in lateral line morphology and differential sensitivities between the two species as a result of their habitat pressures. It also identified increased morphological variation of the lateral line within lacustrine and riverine populations of *G. cotidianus,* which is thought to be a direct result of different habitat and water mechanics. The lateral line mechanosensory system is thought to be important for individual survival as it aids in rheotaxis, predator avoidance and prey location. The study of differences in interspecific morphology may play a role in identifying potential habitat ranges (Vanderpham et al, 2013).

Genetic divergence influences not only the form of the fish but other factors such as growth and reproductive timing which was observed via differential reproductive peaks between populations (Bleackley, 2008). The biological processes previously stated have been noted by Michel et al (2008) as being distinct between populations as a result of different environmental pressures. The fish used for the purpose of this thesis is a lacustrine population sourced from Lake Karapiro, a lake formed from the central Waikato river as a result of the development of the Karapiro power station in 1940 (Withers, 1950). From personal observations, individuals sampled from this population display physical features matching those of other non-diadromus populations of *G. cotidianus* (Michel et al, 2008). This was most apparent by looking at the canals (pores) on the top of the head as the high majority of fish from Lake Karapiro had decreased canals (if any) which was also seen in a study by Michel et al (2008) when comparing the morphology of riverine and lake populations of *G. cotidianus* in the Bay of Plenty.

1.4 Species identification and characteristics of G. cotidianus

Colour- Body and fins are light sandy brown/grey with darker brown irregular blotches across the body to aid in camouflage with the sediments. Cheek and gill plates have dark brown horizontal stripes on them, often 2-3, and dorsal fins often have 1-2 horizontal gold stripes (McDowall, 1994). Juvenile colouration is often lighter with less dark blotches on the body. Male colouration within the genus has been known to darken during the breeding season to dark brown black throughout the body which is the nuptial colour of a sexually reproductive male (Hamilton & Poulin, 1999).

Shape and size- Adult fish range between 40 mm and 160 mm in total length, with lake populations often being smaller in total length than river populations. Body is torpedo shaped with a flat ventral side and relatively broad head tapering to a point at the tail (McDowall, 1990).

Physical Characteristics- A pair of pores can be seen on the head situated between the eyes. The riverine ecotype can be characterised by the presence of extra pores on the top of the head around the crown of the skull that are visible on the adult (McDowall, 1975). The lacustrine or lake dwelling ecotypes are often missing the additional pores, and identification of juveniles may require a microscope to see the small open pores. Dorsal fin ray count for species identification is often seven but occasionally has been noted as having eight (McDowall, 1994).

Behavioural characteristics- *G. cotidianus,* like the majority of the New Zealand native fish fauna, is a relatively shy fish taking cover under weed and rocks and other objects whenever threatened. Slower moving waters such as lakes, ponds and large rivers with current refuge are preferred over faster flows such as high aspect streams and rivers. Males of the species display nest guarding behaviour during spawning seasons, cleaning and prepping a suitable nest site for a mate to deposit her eggs followed by the fertilization and guarding of the eggs until they hatch. Based on average clutch size observations in comparison to female GSI indices, Stephens (1978) suggested that multiple females will occasionally spawn in a nest guarded by the same male. This behaviour is most likely

due to the optimality of the nest which the male is guarding or the fitness of the male.

1.5 Male alternative reproductive techniques

The majority of fish species display an extensive size range of sexually mature reproductive males within a single population, allowing for divergent intraspecific reproductive techniques and alternative male reproductive phenotypes (Taborsky, 1998). Male alternative reproductive techniques (MARTs) are differential methods used by males of the same species within a population for the ultimate goal of reproductive success. Each technique will often include various levels of energetic investment, parental/brood care, territorial or aggressive advances and courtship behaviour (Gross, 1984). It is currently unknown what governs the initiation of a specific reproductive technique by an individual but evidence suggests that factors include maturity, size and sex ratio of the population as well as environmental factors such as temperature and flow (Cargnelli & Gross, 1996).

Animals may either invest energy in order to gain access to a resource directly or they may invest energy in the attempt to gain access by exploiting the investment of others, these are known as comparative reproductive techniques (Taborsky, 1997). Spawning fish display some of the most diverse examples of MARTs in the animal kingdom. Some of these include pirates, female mimics, sneakers and territorial nest guarding males also known as ‘bourgeois males’ (Taborsky et al, 2001). Each technique has slightly different behaviours that define them. Pirates take over the nest site of another established male for a limited spawning period and then leave, resulting in the original male returning and carrying out the brood care (Taborsky, 1994). Female mimics are males that are morphologically and behaviourally specialized to gain access to a guarded nest by appearing as a female whereas sneaker males require the male to leave the nest or be pre-occupied to get past the nest guarding males and gain access to the established nest (Taborsky, 1994).

MARTs have not yet been documented in the *Eleotridae* but commonly occur in the closely related family *Gobiidae* with species such as the common goby (*Pomatoschistus microps*) displaying similar territorial and brood care behaviour as observed in the common bully with the addition of sneaking males (Gross & Sargent, 1985). These sneaking males are smaller in body size than territorial males and because of this, fail to adequately out-compete large males for nesting territory (Magnhagen, 1994). Lack of territory to establish a nest site will result in no chance of reproductive success for the small males, therefore the male must shift to an alternative reproductive technique in order to successfully reproduce that season (Magnhagen, 1994). It is expected that males would engage in behaviour that potentially enables them to achieve some reproductive success (Gross 1982). Therefore, MART's such as sneakers in many organisms, especially fish and bird species, often can be interpreted simply as young, small or inferior males making the best of a bad situation (Kodric-Brown & Brown, 1984).

Depending on the size and coloration of small subordinate males within a population that has nest guarding / sneaker interactions, the sneaker male may double as a female mimic to gain access past the guarding male to the nest (Taborsky, 1994). The female mimic as a MART is similar to the sneaker although it does not require the bourgeois male to leave the nest or be preoccupied in order to access the nest and fertilize the eggs. The appearance of the mimic allows access directly past the male by deception that the male is allowing another female into the nest to spawn (Taborsky, 2008). An example of female mimicry was noted in Japan in the sexually dimorphic freshwater goby, *Rhinogobius sp.* Within a sample of 171 adult fish, three males were identified as being morphologically similar to the female of the species, thus gaining access to a guarded nest and reducing the chances of nest guarding males identifying them as male competition. The gametic investment of the three mimics was significantly higher than the gametic investment of the regular males within the same population, and this suggests the potential for sneaking fertilization in *Rhinogobius* (Okuda et al, 2003). An example from gobies in the genus *Pomatoschistus* discovered that small males with comparatively large

testes found during the breeding season are thought to be indicators of the existence of alternative reproductive tactics such as sneaking behaviour (Magnhagen, 1992).

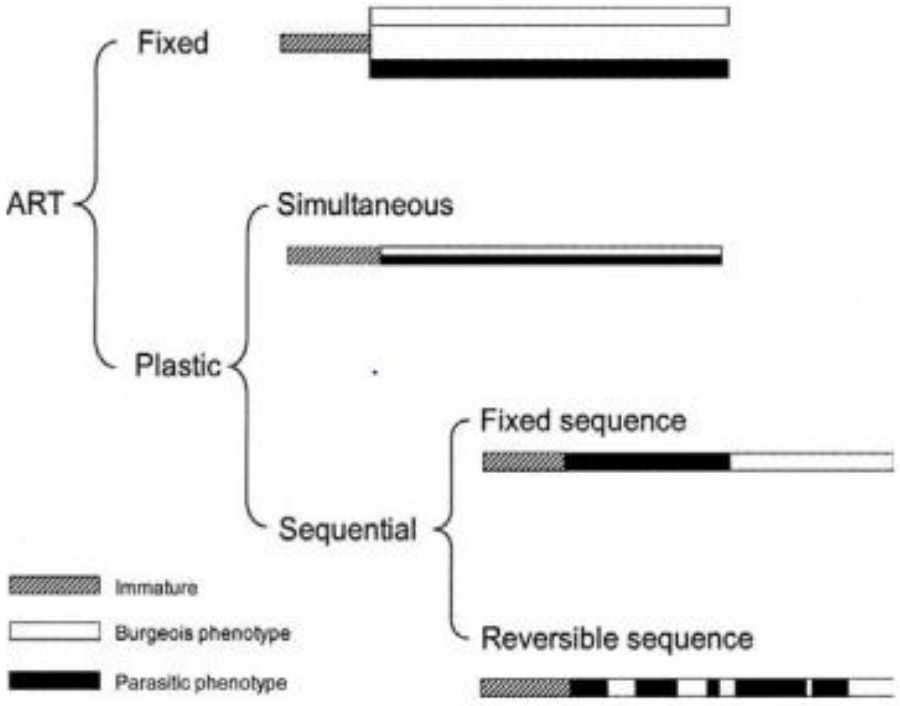

Figure 1.5: Alternative reproductive techniques may be fixed, plastic or sequential depending on species, environment and life phase. Retrieved from Oliveira et al (2008).

MART's have evolved to overcome physical or population based barriers that would normally limit reproduction in a particular species. Fitness can be gained by perusing different reproductive tactics to exploit investment within same sex competition. A fitness advantage to the individual occurs if it is reproductively successful and therefore increases the chances of future reproductive success (Oliveira et al, 2008). Thus, each decision that is acted upon has cascading effects into the future by resulting in changes to physiological state (such as energy expenditure and gonad investment). Therefore, cascading effects will consequently influence the choice between alternative decisions at any given time (Oliveira et al, 2008).

MART's are thought to occur most extensively within the teleost fishes but there are also numerous examples of them occurring in other taxonomic groups such as birds, reptiles, amphibians, invertebrates and mammals

(Oliveira et al, 2008). The vast range of animals that display MART's is testament to the important role they play in reproductive potential and functional evolution of species. The explanations for the existence of MART's across all taxonomic groups can be considered at two logical levels: the proximate factors that give rise to conditions favouring alternative techniques and the ultimate functional consequences that are determining factors in their evolutionary stability (Dunbar, 1982).

1.6 Nest guarding and brood care in Gobiomorphus

New Zealand's *Gobiomorphus* have been documented as displaying nest guarding and brood care behaviours during the spawning season. Males establish and defend territories under, or on top of a rock or fallen tree to be used as a nest (McDowall, 1990) and the males then attract reproductively mature females to spawn in the nest defended by the male (Poulin, 1994). One or more females may spawn in the same nest site guarded by a single male. Spawning is achieved as females deposit a single layer of eggs, often on the underside of the substrate, accompanied by the male following behind fertilizing the newly laid eggs. Males then guard their brood until hatching by driving away the female from the nest post-spawning and protecting the nest area from intruders. Throughout the guarding period the males clean and fan their eggs using a figure eight oscillation with their pectoral fins to draw water into the nest and flush it across the nest supplying oxygenated water to the nest (Hamilton, 1998).

Egg cannibalism is thought to occur commonly in New Zealand bully populations as evidenced by the presence of bully eggs found within the stomachs of adults, often males (Cadwallader, 1975). However, it is not clear whether males eat only their own eggs or the eggs guarded by other males within the vicinity of their guarded territory (Lindström & Hellström, 1993). There are a number of biological costs attributed to the actions of parental care including reduced physical condition, lack of feeding opportunities, increased susceptibility to predation and greater energy expenditure compromising future reproductive success. A percentage of these costs can be compensated by consuming a portion of the eggs

which provides the male enough energy to continue the parental care and therefore increases the chances of the rest of the brood successfully hatching. This is known as filial cannibalism. A non-energetic motivation for partial filial cannibalism may be the removal of diseased, malformed or unfertilized eggs from the nest to promote the health of the rest of the brood (Manica, 2002). The degree of investment by the parents is thought to be influenced by the expected reproductive success of the brood including the presence of predators in the environment, size, number and age of the eggs in the nest and the size, age and health of the parents (Stott & Poulin, 1996).

Brood care and parental investment greatly increases the proportion of progeny that survive the egg development phase through to the hatching phase. This is achieved by increasing oxygen availability through fanning, reducing predation through guarding and preventing disease and infection through cleaning and maintenance of the nest. This reproductive technique is common in egg laying fish as the number of eggs that can be produced at one time is limited by the size of the fish and the energetic resources that are used in egg production (Sargent et al, 1987). Therefore, rather than investing in laying an extensive numbers of eggs in the hope that a percentage will survive without parental care, the energetic investment is put into parental care of fewer eggs in the hope that most of the clutch will survive (Perrone & Zaret, 1979).

Broadcast spawning involving large numbers of eggs often occurs when there is a lack of predators to consume eggs and juveniles as parental care predominantly protects against predation. This involves high investment in gonads by both sexes with reduced fertilization rate and reduced chance of predation. This is common in fish such as the *Clupeidae* (herrings) and *Engraulidae* (anchovies) where vast numbers of eggs are produced, with only a portion surviving but no parental care involved (Perrone & Zaret, 1979). It has been reported that egg size increases in relation to parental care quality and duration allowing for increased hatch potential as seen in the fish families *Cichlidae, Percidae, Salmonidae* and *Centrarchidae* (Gross & Sargent, 1985). In general, fish

species that are known to provide parental care also produce eggs that are larger in size than species that broadcast spawn or provide no parental care (Duarte & Alcaraz, 1989, Kolm & Ahnesjö, 2005).

1.7 Seminal vesicles and testes – Form and function

Seminal vesicles are accessory reproductive organs also known as sperm duct glands found in males of at least 7 families within the *Teleostei* including the *Gobiidae* (Lahsteine et, al. 1992). The organs are paired and elongated with a lobular shape that changes slightly between males of different sizes and ages (Nagahama, 1983). During spawning seasons, seminal vesicles increase in size and weight as production of seminal fluid increases. The fluid produced is predominantly mucin, a viscous secretion made from sialoglycoprotiens (Mazzoldi et al, 2005). This increased production occurs in preparation for fertilization and acts as a slow release, sperm laden gel which is spread over clutches of eggs by the male. The viscosity of the secretion is dependent on the proportion of mucin present in the seminal fluid, and this is evident in species known to display male alternative reproductive tactics. For example, nest guarding males produce a secretion higher in mucins with a longer lasting duration of sperm availability but less sperm density than that of a sneaker male. The secretions of a sneaker male are less viscous with a high sperm density for optimized fast flooding of an egg clutch but short duration due to reduced levels of mucin (Mazzoldi et al, 2005).

These characteristics suggest two main male reproductive mechanisms, with bourgeois male traits guaranteeing a lower steady supply of sperm for hours compared with the sneaker males releasing a high density and quickly diffusing number of sperm that out-competes the sperm of the bourgeois male for a short period of time.

Male testes have a similar form to seminal vesicles as paired elongated organs attached to the dorsal body wall with a lobular shape. They are often a cream to yellow colour with a large number of blood vessels and a changing organ diameter (depending on the season) in relation to

spawning and rate of reproduction. The internal wall of each lobe contains somatic cells as well as cysts of germinal cells where the sperm develops via the process of spermatogenesis. The germ cells within each cyst are approximately at the same developmental stage with maturation occurring around the same time. Once mature, cysts rupture releasing the mature germ cells in the form of sperm into the lobular lumen in preparation for fertilization (Cinquetti & Rinaldi, 1987). When sperm is expelled during fertilisation it is combined with the mucins produced in the seminal vesicles, resulting in a complete seminal fluid for egg fertilization. The consistency and sperm density of the seminal fluid is unique to each individual male depending on age, size, environment and male alternative reproductive technique used by the fish, resulting in high sperm competition within communities (Marconato et al, 1996).

1.8 Thesis aims and hypothesis

Gobiomorphus cotidianus is a member of the family *Eleotridae* which displays a number of morphological and behavioural reproductive similarities to the closely related *Gobiidae* family. Given that there is extensive international literature investigating the diversity and functionality of MART's within the goby family (Magnhagen, 1998; Rasotto & Mazzoldi, 2002; Malavasi et al, 2003; Lehtonen & Lindström, 2008; Marentette et al, 2009) but no research into the reproductive strategies of the New Zealand *Eleotridae,* there is an obvious need for initiation of research in this field.

The aim of this research was to identify evidence to support or deny the possibility of MART's in this genus. Based on supporting physiological evidence in related families (Locatello et al, 2007; Oliveira et al, 2008) I hypothesised that males who invest energy in nest guarding and brood care, will likely invest more heavily in seminal vesicles than gonads. On the assumption that MART's occur in the genus *Gobiomorphus*, I expect non-nest guarding males to invest more heavily in gonads than seminal vesicle on the basis of increased sperm competition between the

alternative reproductive tactics and the redundancy of seminal vesicles due to lack of nesting.

1.9 Thesis Outline

This thesis comprises of four main chapters in the classical scientific layout of introduction, methods, results and discussion. Chapter one reviews what is already known about the general biology and life history of the gobiomorphus in New Zealand. It also provides a background into the distribution and functionality of MART's in members of closely related families as well as setting the thesis aims and general hypothesis of the research. Chapter two presents each step of the methodology used for all aspects of this study including capture, dissection, design construction and analysis. Chapter three describes the results gained from each aspect of the study, whilst Chapter four discusses the relevance of the results in comparison to other research and critically evaluates the behavioural experiment and suggests directions for future research. Literature cited throughout this document is acknowledged in full in the references section at the end of this thesis.

Chapter Two - Field Collection Methodology

2.1 Study site

Common bully were collected monthly from Lake Karapiro commencing in December 2013 and concluding in January 2015. Fish were collected using Gee minnow traps, by placing three string lines which each held 10 traps attached at 2 m intervals. Traps were set over the side of a boat towards the centre of the lake in order to cover a range of depths. Gee minnow string lines were attached to trees overhanging the lake shore. Lines were secured with a brick at the free end to reduce movement of the traps. To help reduce bycatch, minnow traps were set in the evening and collected first thing the following morning. Each month, 80 - 120 individuals were collected depending on catch numbers.

Once collected, traps were emptied into a 100 L fish bin filled to 1/3 capacity with lake water. Non-target species were returned to the lake as well as common bullies that were smaller than 3 cm total length. This was to ensure that fish being collected were reproductively mature (Stephens, 1982). Fish were transported back to the University Of Waikato's Aquatic Research Centre, where they were transferred into three 250 L fiberglass tanks containing some of the lake water that they were transported in and topped up to 200 L with dechlorinated tap water.

Air bubblers were placed within each tank to ensure the water remained well oxygenated and to provide water flow within the tank. In addition, artificial seawater (Crystal Sea Marinemix salt; Marine Enterprises International, Baltimore, Maryland, U.S.A) was added to the tank at a ratio of 1:10 to help prevent fungal infections such as white spot and fin rot from occurring whilst in captivity. The artificial seawater also helped to reduce stress on the fish as a result of capture handling and transportation. When fish were maintained other than for dissection, on every second day, 1/3 of the tank water was emptied and replaced to help maintain stable nitrate, ammonia and pH levels which can reduce fish stress and prevent disease or mortality (Ling, 2006b).

2.2 Dissection of gonads

On return to the laboratory, bullies were left in the holding tanks for 24 h to evacuate the gut contents that might contribute to varience in somatic weights and were then euthanized with an overdose of 2-phenoxyethanol at a dosage of 0.5 mL/L. Fish remained in the solution for a period of 10 min. This dosage is twice the recommended anaesthetic dosage for 2-phenoxyethanol in accordance with University of Waikato standard operating procedure 6, Euthanasia and Anaesthesia of Fish (Ling, 2006a). Once euthanized, individuals were removed from the solution and blotted dry with paper towels to remove any excess water. Emphasis was placed on the head as water gathers under the gill cavity and in the mouth. Once dry, the total length and standard length of the fish (± 1 mm) were recorded using a standard metric fish board. Total weight (± 0.001 g) was measured on a top loading balance.

Fish were transferred to a wax dissection board where the dorsal fin rays (DFR) were counted to help confirm species identification before being placed ventral side up and pinned through the lower jaw and at the base of the anal fin to prevent movement during dissection. An incision was made using a size 10A scalpel from the anal opening to the pectoral fin girdle, exposing the gut cavity. The intestine was cut from the posterior end of the gut cavity and pulled back to the anterior end of the fish, exposing the reproductive organs underneath. The cavity was then filled with water to suspend the reproductive organs helping to prevent damage during removal.

If the specimen was female, an incision would be made from the base of the pectoral fin towards the dorsal fin on each side of the fish allowing easy access to the ovaries and reducing the risk of puncturing the ovary membrane when removed. Each end of the ovary was clamped with fine nose forceps to disconnect the ovary membrane from the body allowing the ovary to be removed without the eggs spilling into the gut cavity. Once both intact ovaries were removed, they were blotted dry with paper towels and weighed (± 0.001 g). Ovaries were then placed in 1.5 ml centrifuge

tubes containing 0.5ml - 1ml of 10% formalin, and set aside for the possibility of future fecundity testing.

If the specimen was a male, the seminal vesicles and testes were located. These organs lie either side of the bladder and to remove, forceps were clamped underneath the bladder, seminal vesicles and testes and removed as a whole. The seminal vesicles and testes were then detached before being blotted dry and the bladder discarded. The organs were then weighed separately (± 0.001 g). Gonad mass indices, male and female gonadosomatic index (GSI) and seminal vesicle somatic index (SVSI), were calculated with respect to somatic weight (total weight – gonad and seminal vesicle weights).

2.3 Behavioural Experiment

2.3.1 Laboratory preparation

A behavioural breeding experiment designed to observe preferences of mate choice, nest site selection and nest guarding activity was undertaken in December 2015 following the observation of a peak in gonadosomatic index in both males and females from Lake Karapiro. Fish were caught using the same methodology as for the seasonal sampling for GSI and SVSI indices, with 120 fish used as active trial subjects with the proportion made up of 60 males and 60 females. Fish were held in three 250 L holding tanks for one night before being separated by sex into separate 250 L tanks.

Fish were sexed twice using 3 different methods; the first method used was visual, based on the presence or absence of enlarged ovaries which are visibly yellow when looking at the underside of the fish during the summer spawning season. Fish that were obviously gravid with a swollen yellow abdomen from the ripe ovaries were placed into the female only tank. The remaining fish which could not be visually identified were then lightly anesthetised using a solution of 2-phenoxyethanol at a dosage rate of 0.25 mL/L. Fish were left in the solution for two minutes or until they were lethargic and easily handled. With exposure to the anaesthetic, male

fish often changed colour turning a dark greyish black. These darker coloured males were removed from the anaesthetic and placed into the male only tank to recover. The remaining fish that did not display obvious yellow ovaries or change colour in the solution were sexed by the shape of the genital papilla with the males being narrow and showing a more tapered point whereas the females were broader with a rounded point.

Once sexed, the common bully were categorised by size and each sex was split into two size groups, regular and large. Regular fish were classed as being between 45 mm and 60 mm total length with the large fish classed as being between 70 mm and 90 mm total length. These measurements were taken whilst the fish were anesthetised to reduce stress as stress related injuries can be caused by excess movement on the measuring board.

2.3.2 Experimental construction

The experimental set up was constructed in four 1500 L tanks situated outside of the University of Waikato Aquatic Research Centre. Within each of the 4 tanks, ten 9.5 L plastic buckets were placed upside down which had the handles and bottoms removed. Each bucket contained 15-20, 3 mm diameter holes melted through the sides of the bucket to allow water circulation. To act as a lid we cut the bottom third off another 40 buckets removing an 8 cm diameter hole from the centre of the bottom of the bucket. This piece was then covered with 2 mm mesh that was glued in place (Figures 1 & 2). This allowed light to enter the bucket, prevented the fish from jumping out, and provided easy access to check inside the buckets each day. The lids fitted onto the upside down buckets creating a secure arena in which the experiment could be undertaken. Each system was weighted down with four lead fishing weights placed in the rim of the upside down buckets. This prevented fish from escaping under the buckets, and helped reduce bucket movement and floating during water changes. Further, the weights allowed observation to be undertaken by keeping the buckets grounded while the lids were removed to view the fish inside. Each bucket contained two halved terracotta plant pots to provide

nesting habitat. The pots used were either 9 or 13 cm in diameter providing either smaller or larger nest sites, respectively.

Figure 2.1: Breeding experiment displaying 4 experimental treatments each containing 10 breeding arenas

Figure 2.2: Breeding experiment showing how arenas were positioned and weighted down using small weights, the blue section is the removable lid of the arena

The water level within the four troughs was filled to half way up the sides of the buckets or approximately 500 L per trough. A 20% water change was carried out once a day during the course of the experiment to help reduce nutrient build up and maintain water quality and clarity. Water temperature readings were taken from each trough at approximately the same time each day before the water change took place, this was done using a YSI EC 300 conductivity meter. This was done at approximately 3 pm to identify the maximum temperatures that the troughs would likely reach throughout the experiment. Each of the troughs were fitted with air bubblers to maintain satisfactory dissolved oxygen levels and to promote water movement through the buckets. Further, the troughs were fitted with custom made metal framed white shade cloth covers designed to help reduce temperature spikes throughout the day and keep debris from falling

into the tanks. These covers also delayed the production of algae and periphyton growth within the experiment, reducing the degradation of water quality and clarity.

2.3.3 Experimental treatments

The experimental treatments were designed to test three main factors, mate choice, nest site preferences and male nest guarding. To address this we designed four treatments of reproductive interactions with 10 replicates of each. The control was treatment A and contained 1 regular sized male and 1 regular sized female per replicate (bucket). This was designed to give a baseline for the time it took for males to establish a nest and for females to spawn. It also allowed the average clutch size for a single female to be acknowledged and the guarding behaviour of a male fish without the threat of male competition, and was designed to determine whether males selectively choose either the larger or smaller nest pot. Treatment B contained one regular sized male, one regular sized female and one large female. This was designed to identify if multiple females spawn in a single nest or if the larger and presumably alpha female spawns and either actively or passively prevents the subordinate from spawning.

Treatment C contained one regular sized male, one large male and one regular sized female. Having two different sized males in the bucket provides females with a choice of spawning with either the larger or the smaller male or lay a percentage of eggs in both nests. Encompassing two different sized males into this treatment also allows for observations to be drawn on male territories in relation to nest size selection. Close proximity habitat of two different size nests allows for male choice of optimal nest size and provides possible correlation of links between male size and fitness and nest size intraspecific territoriality. Treatment D contained one large male and one regular size male as well one large and one regular sized female. This treatment was designed to observe differences in female mate choice as well as nest size selection. Nest size selectivity by males and correlation to male total length and presence of nuptial

coloration can be identified by the presence of males guarding their chosen nests. Differences in female total length is important to observe if total length is correlated to total length of the chosen mate or if length is not the most important factor in mate selectivity. All treatments contained both large and small pot nests to test for selectivity of nest site.

Data was collected at the same time each day for a period of 7 days after the experiment began, the lids on each of the buckets for every treatment were removed and the fish were then left to settle for 15 minutes. Observations were noted on the position of the males in each bucket in respect to the nest pots. Guarding behaviour was evident as even when disturbed, males did not move from their position either on top of the pot or directly in front of the opening of the pot. If males were guarding it was noted what size pot they were guarding and for what duration in days that they guarded for.

After the position observations for each of the males was completed, each pot was checked both inside and out for the presence of eggs. If eggs were present then it was recorded what pot they were on and if they were scattered or spawned in a clutch. If spawned in a clutch the size of the length and width of the clutch was measured and if scattered a rough estimate of eggs were counted and recorded.

Figure 2.3: large male nest guarding the top of the small nest pot prior to female spawning

2.4 Indices and Statistical Analysis

Gonad mass indices, male and female gonadosomatic index (GSI) and seminal vesicle somatic index (SVSI), were calculated with respect to somatic weight (SW = total weight – gonad and seminal vesicle weights). Fish condition was calculated as:

K = (100000 x SW)/(SL3) - where SW = somatic weight in g and SL = standard length in mm.

Relationships between gonadosomatic indices of males and females with respect to fish total length were analysed by correlation and linear regression in Graphpad Prism and tested for deviation of the slope of the regression line from 0 using the F test. Selectivity of nest and mate choice was examined using Fisher's exact test in Graphpad Prism. Statistical significance for comparative reproductive investment of experimental males was determined using Student's T-test. Monthly data for GSI, SVSI and SVSI/GSI for males and GSI for females were analysed by one-way ANOVA to test for significant differences in mean values and for heteroscedasticity (differences in variance) using Bartlett's test in GraphPad Prism.

3.1 Seasonal variance in gonadal development (GSI)

Trends in seasonal gonad development are presented by the use of gonadosomatic indices (GSI %) and are shown in Figure 3.1 for males and Figure 3.2 for females over a period of 14 months from December 2013 to January 2015. Male mean GSI ranged from a low of 0.58% in January 2014 to a peak of 1.23% in November 2014.

Male common bully exhibited much smaller seasonal changes and invested significantly less energy into gonadal development compared with females whose mean GSI varied from a low of 0.97% in March 2014 to a peak of 9.01% in September, although some individuals developed ovaries that constituted 22% of their somatic body mass. Increased gonadal mass in males in preparation for spawning was delayed by one month compared with that in females. Both males and females displayed large variability in gonadal mass during the spring spawning season.

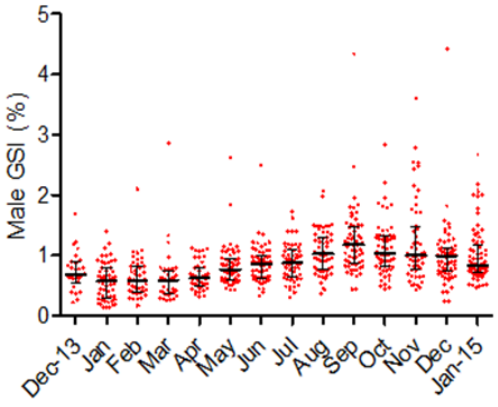

Figure 3.1: Common bully male gonadosomatic index (GSI) percentage, sampled monthly from December 2013 to January 2015. Horizontal bars represent median ± interquartile range.

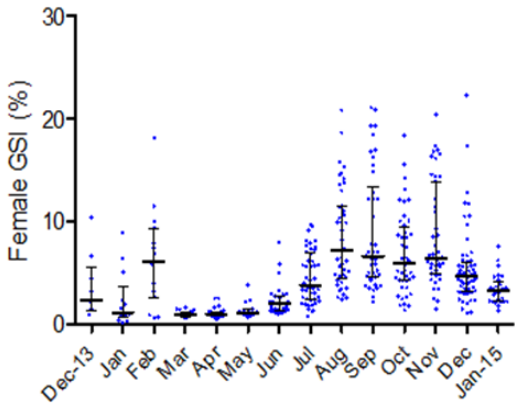

Figure 3.2: Common bully female gonadosomatic index (GSI) percentage, sampled monthly from December 2013 to January 2015. Horizontal bars represent median ± interquartile range.

3.2 Seasonal variance in male seminal vesicle development (SVSI)

Figure 3.3 shows the seasonal variance in seminal vesicle somatic index (SVSI %) over the 14 month sampling period from December 2013 to

January 2015. The seminal vesicle mass of males showed a more pronounced seasonal variation compared with male GSI with mean SVSI varying from a low of 0.16% in May to a peak of 1.75% in October. Individuals displayed large variation in SVSI mass during the spring spawning season. Male GSI increased gradually coming onto the spawning season, whereas SVSI remained low over the winter period with a sharp increase in spring which slowly declined throughout summer. SVSI showed a more defined correlation with natural spawning season compared to GSI.

Mean values for GSI, SVSI and SVSI/GSI in males and GSI in females differed significantly between months with significant increases occurring during the spring spawning season (ANOVA, $P<0.0001$ for all three parameters). Furthermore, the variance in each measure also increased significantly during this time (Bartlett's test, $P<0.0001$ for all). The same was observed for female GSI with significant increases in mean GSI and variance during the spawning season (ANOVA, $P<0.001$; Bartlett's test, $P<0.001$).

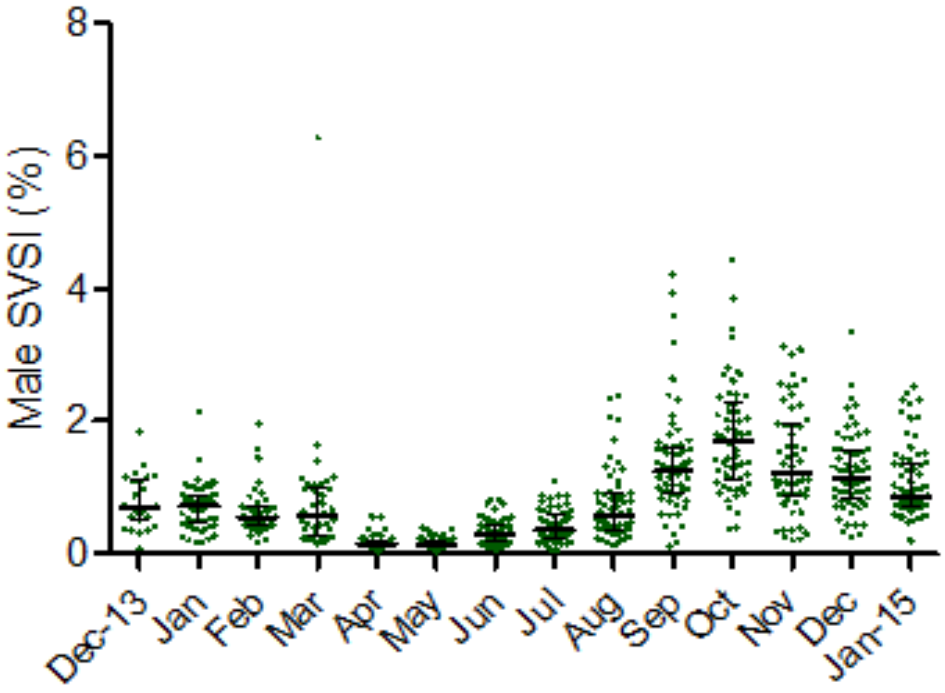

Figure 3.3: Male common bully seminal vesicle somatic index (SVSI) percentage, sampled from December 2013 to January 2015. Horizontal bars are median value ± interquartile range

3.3 Seasonal variance in male SVSI/GSI ratio

The male SVSI/GSI ratio plotted monthly from December 2013 to January 2015 in Figure 3.4 shows the ratio of relative weight of the seminal vesicles and gonads in order to identify seasonal changes in the investment of energy in both male reproductive organs. The SVSI/GSI ratio followed a very similar trend to that of the SVSI values due to much greater seasonal variation in SV development compared with the testes.

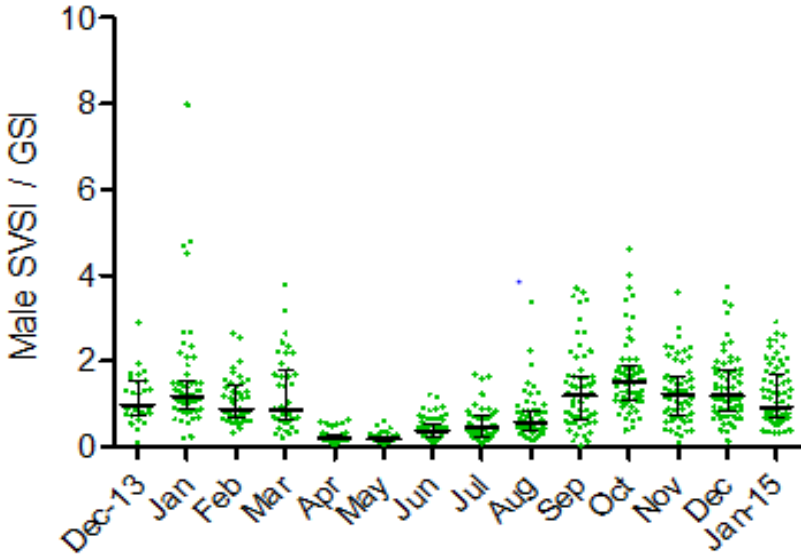

Figure 3.4: Male common bully seminal vesicle somatic index (SVSI) gonadosomatic index (GSI) ratio, sampled from December 2013 to January 2015. Horizontal bars represent median ± interquartile range.

3.4 Male and female GSI / length correlations

Relationships between gonadosomatic indices and fish total length were analysed by pooling data from all months encompassing reproductive maturity (August to December for females and September to January for males). Although it is clear from the data in Figure 3.5 that males of all sizes displayed a range of values for GSI during the reproductive season there was a significant negative correlation between male fish total length and GSI (P=.0.0086, Pearson r = -0.148, r2 = 0.0219, F=6.99) with smaller males showing a tendency for relatively larger testes.

In females there was no significant correlation between GSI and fish total length although it is apparent from the data in Figure 3.6 that larger fish typically do not exhibit GSI values exceeding 10%. The degree of correlation varied greatly between males and females due to the differential investment in GSI (%) of each sex. Female GSI was significantly negatively correlated with fish condition (P=0.013, Pearson r = -0.163, r2=0.0265, F=6.25) although condition was not significantly correlated with fish total length.

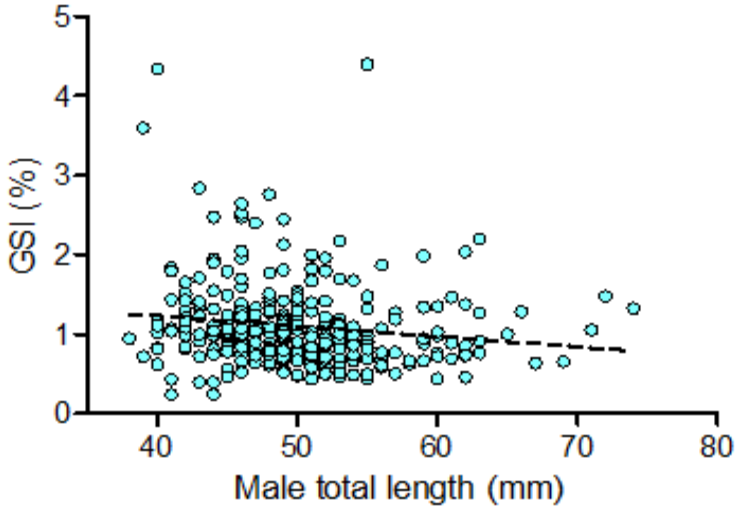

Figure 3.5 Male common bully gonadosomatic index (GSI) percentage in relation to fish total length (mm) over a 5 month sampling period from September 2014 to January 2015. Trend line determined by linear regression

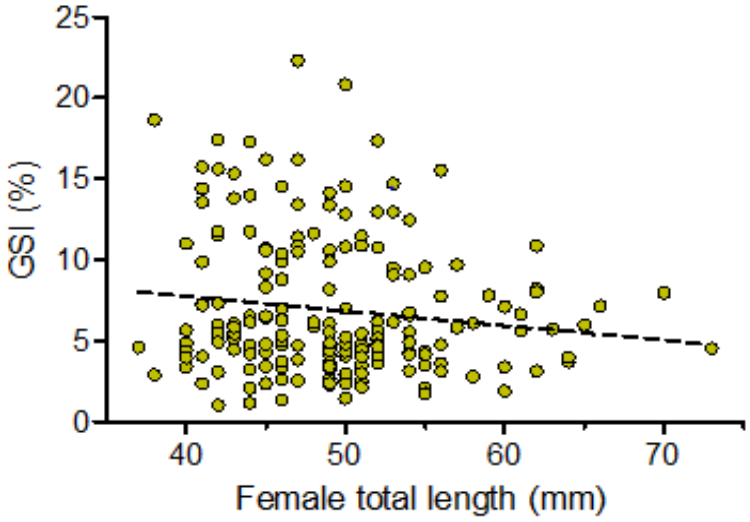

Figure 3.6 :Female common bully gonadosomatic index (GSI) percentage in relation to fish total length (mm) over a 5 month sampling period from August 2014 to December 2015. Trend line determined by linear regression.

3.5 Male SVSI/GSI vs length

In contrast to male GSI, there was a significant positive correlation between SVSI/GSI ratio with male fish total length (Figure 3.7; P<0.0001, Pearson r = 0.251, r2=0.0628, F=20.91). Although fish of all sizes showed considerable variation in SVSI/GSI ratio, larger fish were much more likely to have relatively larger seminal vesicles and presumably therefore more likely to be nest guarding males. Neither GSI nor SVSI/GSI in males was correlated with fish condition.

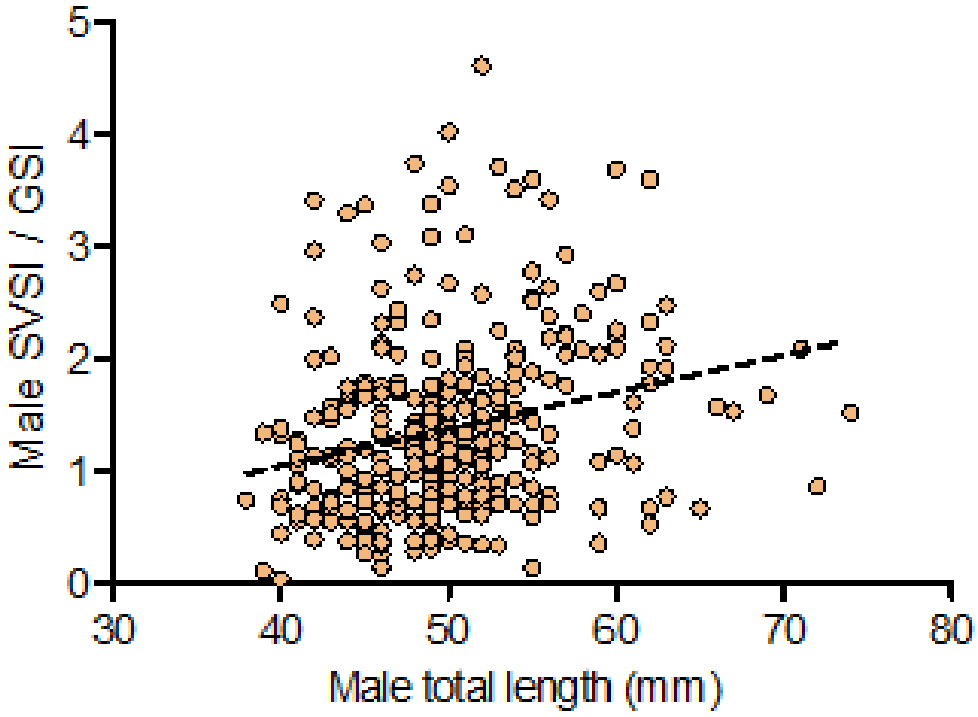

Figure 3.7: Male common bully seminal vesicle somatic index (SVSI) gonadosomatic index (GSI) ratio, plotted over fish total length (mm). Trend line determined by linear regression.

3.6 Male and female length weight relationship

Comparative length weight relationships for male and female common bully are shown in Figure 3.8. Small males tended to be heavier than females but the length weight relationship for both sexes became more even as fish become larger.

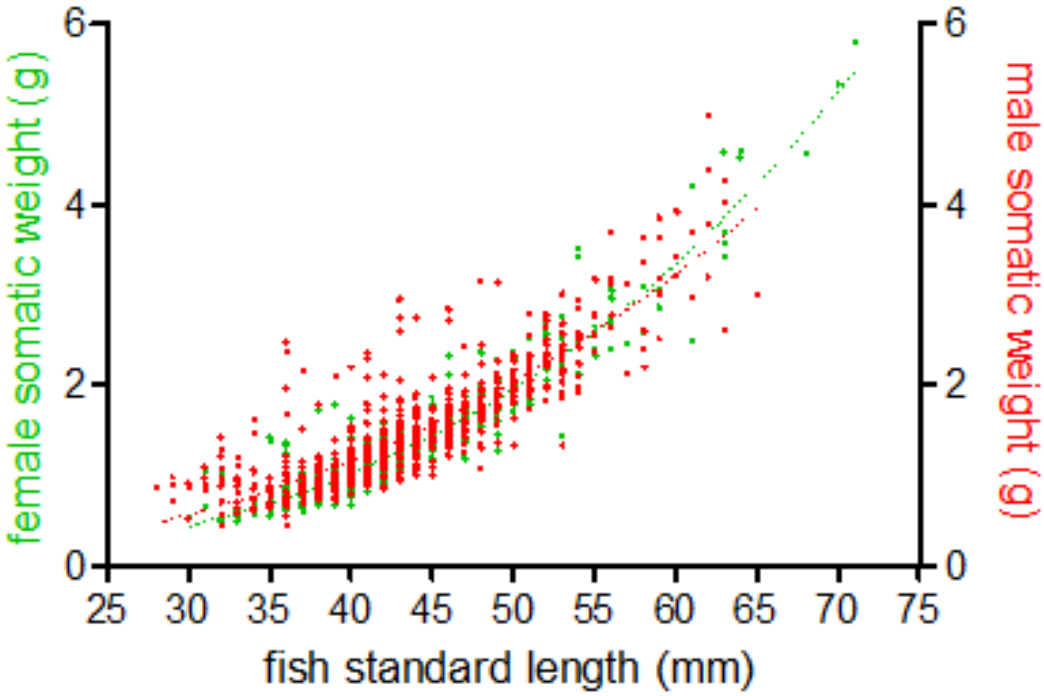

Figure 3.8: Common bully male and female individual somatic weight measured in grams, plotted against the individual standard length of the fish measured in millimetres. Trend line determined by non-linear regression log log relationship.

3.7 Male and female somatic condition index

The somatic condition index for both male (Figure 3.9) and female (Figure 3.10) common bullies was determined using the equation:

K = (100000 x SW) / (SL3) - where SW = somatic weight in g and SL = standard length in mm.

Somatic condition represents the non-reproductive body mass and potentially represents the energy resources available for investment in reproduction. Both males and females showed relatively constant somatic condition throughout most of the year but exhibited much greater variation in somatic condition following the reproductive season with many individuals significantly increasing their somatic condition.

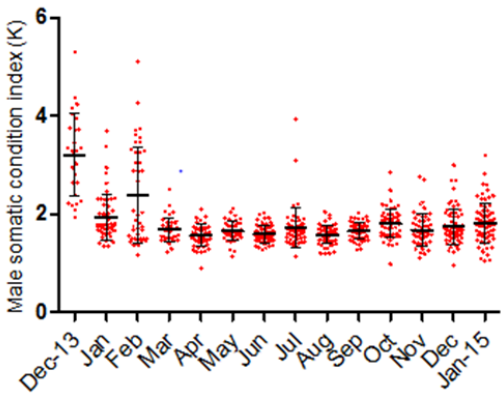

Figure 3.9: Male common bully somatic condition index from December 2013 to January 2015. Horizontal bars represent median ± interquartile range.

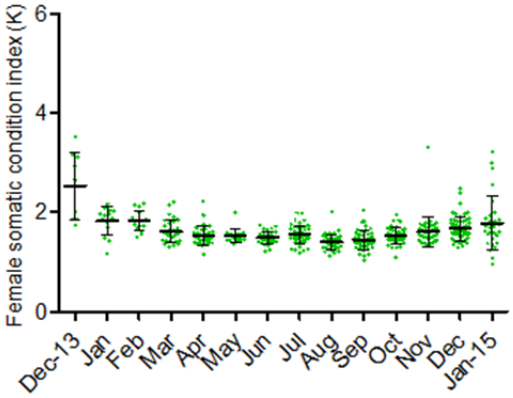

Figure 3.10: Female common bully somatic condition index from December 2013 to January 2015. Horizontal bars represent median ± interquartile range.

3.8 Catch sex ratio

The catch sex ratio throughout the sampling period of December 2013 to January 2015 was dominated by males as the percentage of males in each monthly catch never dropped below 52 percent. Peak male catch percentage was 90% in January of 2014 (see Figure 3.11). Female catch percentage increased leading up to the breeding season and remained relatively stable throughout late winter and spring. Decline in female catch percentage began to occur in early summer, towards the end of the spawning season.

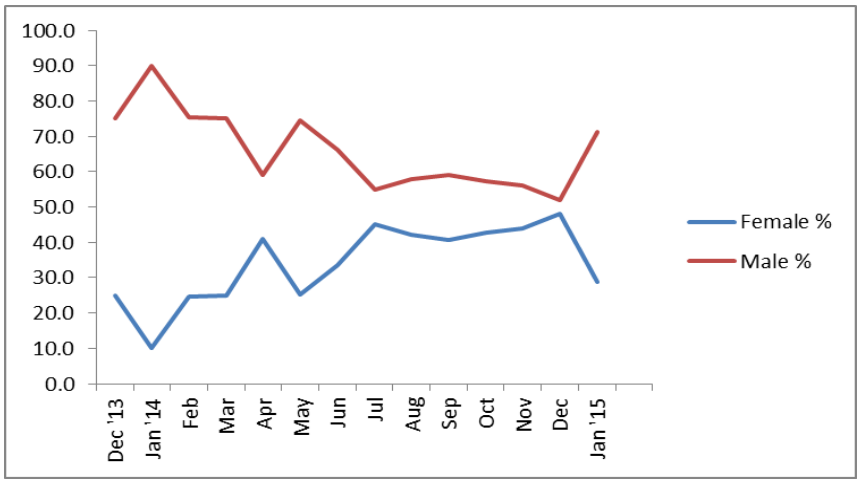

Figure 3.11: Percentage of male and female common bully in catch throughout monthly sampling beginning in December 2013 and commencing in January 2015.

3.9 Nest selectivity of males

Obvious nest guarding was displayed in 14 of the 40 nest arenas during the week of the trial and 11 of the guarded nests were large pots compared with only 3 guarded small pots. However, this sample size was too small to provide a statistically significant result (Fisher's exact test, P=0.237).

Where two males (one large, one small) were present in the same test arena, 10 out 20 arenas displayed nest guarding activity during the trial. Only three arenas displayed guarding of the smaller pot and with two of these guarded by the smaller of the two males. Two larger pots were also guarded by small males but in both cases the larger males had died in these arenas.

These results provide tantalising evidence that males may select large pots as preferable nest sites and that these prime nest sites are dominated by the larger males. Unfortunately, high mortality due to fungal infections and limited spawning activity during the trial did not provide any evidence for mate or nest site selectivity by females.

3.10 Comparitive reproductive investment in experimental males

All of the males that survived the week long breeding exeriment were disected post-experiment. Non-nest guarding males showed relativley equal reproductive investment of gonads and seminal vesicles (as seen in Figure 3.12). Comparitivley, males that displayed obvious nest guarding behaviour throughout the experiment showed increased investment in seminal vesicles compared with gonads.

A Student's T-test was run to determine statistical significance between nest garding and non-nest guarding males for GSI, SVS and SVSI/GSI. GSI AND SVSI T-tests showed results to be insignificant with a GSI P-value of 0.1576 and SVSI P-value of 0.0723. However SVSI/GSI was significant with a P-value of 0.0052

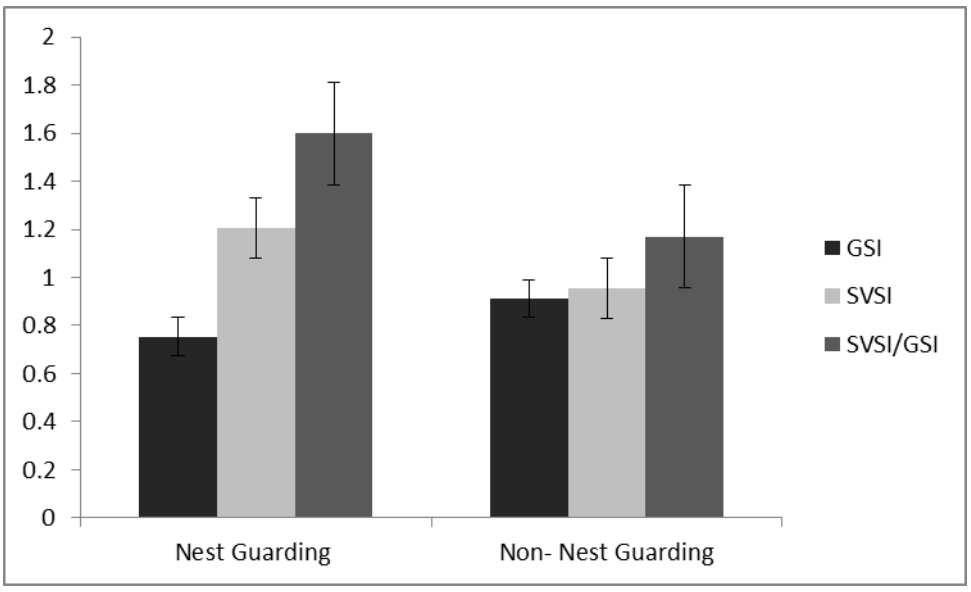

Figure 3.12: Mean reproductive investment in seminal vesicles and gonads of male common bully under experimental conditions. Horizontal bars represent mean ± standard error mean.

3.11 GSI in relation to fish condition

Neither male nor female GSI (%) showed a significant correlation with fish condition (K) although there are clear differences in trends. Male fish condition (K) (Figure 3.13) shows a positive correlation with GSI (%) compared with female fish condition (K) (Figure 3.14) which displays a negative correlation and very little variance in female fish condition compared with the males.

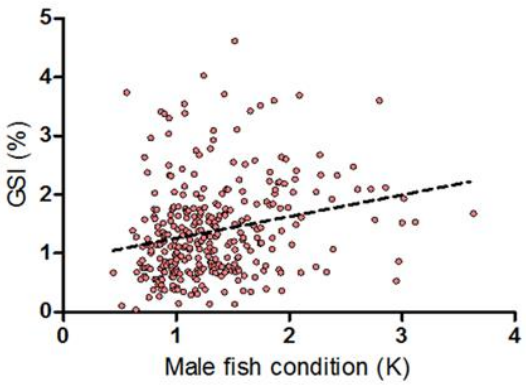

Figure 3.14: Male fish condition (K) plotted against gonadosomatic index percentage. Trend line determined by linear regression

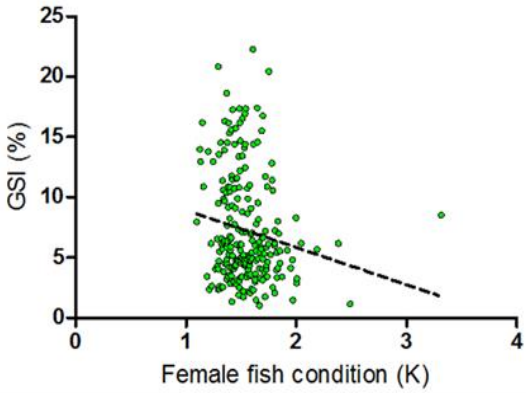

Figure 3.15: Female fish condition (K) plotted against gonadosomatic index percentage. Trend line determined by linear regression.

Chapter Four – General Discussion

The overarching aim of this thesis was to investigate the reproductive investment of the male common bully *Gobiomorphus cotidianus* in order to potentially identify the possibility of multiple alternative reproductive techniques within the species. Whilst there is extensive supporting literature for the presence of multiple alternative reproductive techniques (MART's) in the closely related family *Gobiidae* (Magnhagen, 1992; Taborsky, 1994; Okuda et al, 2003; Mazzoldi et al, 2005) there is no current supporting literature that suggests that MART's occur within the family *Eleotridae*. Previous reproductive and behavioural research on the New Zealand *Gobiomorphus* indicates behavioural and morphological similarities that can be compared between bullies and species that actively portray MART's (Magnhagen, 1998; Rasotto & Mazzoldi, 2002; Malavasi et al, 2003; Marentette et al, 2009 & McDowall, 1990), prompting this specific investigative research on the most common of the New Zealand *Gobiomorphus, G. cotidianus*.

Male common bully have been observed cleaning and preparing a nesting site as well as attracting a female or females to the site in preparation for spawning (McDowall, 1990). Once the eggs have been deposited, the male oxygenates the nest as well as cleaning and guarding the clutch against other males and predators thus, investing energy in ensuring that the clutch has the best possible chances of hatching. This is an example of the only male reproductive investment strategy observed within the New Zealand *Gobiomorphus,* however little research has focused on this aspect of reproduction resulting in most of the noted observations occurring as non-target data of other research projects involving *Gobiomorphus.* The data and knowledge gained through the research and production of this thesis will aid in filling gaps in the basic biological knowledge of the common bully and hopefully prompt further research in this area of reproductive science in New Zealand freshwater fish.

4.1 Gonadosomatic Index

The GSI is the calculation of the reproductive organ mass as a proportion of the total somatic mass. Male and female GSI showed distinct differences in seasonal variation throughout the course of 14 months from December 2013 to January 2015. Male GSI (Figure 3.1) remained relatively stable with little variation during autumn and winter months but a significant increase was observed throughout spring and summer. The increase in gonad size throughout spring and summer is consistent with the onset of the spawning season. Male GSI during the spawning season showed significant variability with a small portion of the sampled males displaying notably larger gonads than the rest of the population. Although this occurred throughout the entire sampling period, the variance increased in the spawning period. This small differentiation may be an indicator for the presence of alternative reproductive techniques within the population as it was originally hypothesised that males potentially displaying sneaker behaviour would have larger gonads and smaller seminal vesicles than nest guarding males.

Female GSI (Figure 3.2) displayed a more pronounced seasonal trend compared with male GSI (Figure 3.1). From December 2013 to February 2014 female GSI was highly variable, this variability would most likely be affected by the low numbers of females caught during this three month period. Throughout March, April and May, female GSI dropped down to minimal weight with decreased variability. This period of gonadal dormancy is likely indicative of a shift in energetic investment with significantly less energy consumed by the development of reproductive organs after spawning. Energy that would have previously been invested in the reproductive organs would most likely be consumed in somatic growth and repair as Stephens (1978) notes that growth of adult common bully was most rapid throughout late autumn and winter when reproduction does not occur.

The average GSI for both males and females was calculated for the 14 month sampling period, this showed the relative energetic investment in gonads by each sex. Male average GSI was 1.07 compared to female

average GSI which was more than five times higher at 5.65. These figures can be used as an example to compare the two different methods of gonadal investment portrayed by the parents (Jonsson et al, 1991). Female bullies have not been observed displaying any form of brood or parental care; after spawning they often leave or are chased from the nest site by the male. Therefore, to ensure optimal reproductive success, the females invest their reproductive energy in the size, quantity and quality of their gametes as opposed to parental care. The males however only invest a portion of their reproductive energy into the quality and quantity of gamete production with the balance thought to be invested in brood care and nest defence (Immler et al, 2004). Males therefore have considerable energetic reserves compared to females that can then be invested in the development of seminal vesicles and activities associated with cleaning and guarding both the nest site and the egg clutch.

The results gained from the 14 month sampling period show that males invested on average 18.95% of the reproductive investment of females in gonad development and production and 21.96% on seminal vesicle development.

This was calculated by converting the average female GSI for the year to a percentage assuming 100% of reproductive investment is invested in physiology (gonad and egg development) as females do not invest reproductive energy on guarding or nest building behaviour. Therefore, average male GSI and SVSI for the year were also converted to a percentage for comparison of physiological investment against females. By adding together the male reproductive investment in SVSI and GSI, we get a total of 40.91% of male reproductive investment invested in physiology (gonad and seminal vesicle development as well as sperm and seminal fluid production) compared to the 100% physiological reproductive investment of the females. The remaining 59.09% of male reproductive energy is assumed to be invested in parental care and nest guarding behaviour.

4.2 Gonadosomatic Index and length

Both male and female GSI declined with increasing fish length. This data suggests that gonad size does not increase exponentially with fish body size and that gonad development ceases to occur past an optimal point in which energetic investment in gonadal development no longer benefits the costs of increasing size and production (Stoltz, 2005). However somatic growth still occurs past this point resulting in a decline in the GSI relation to total length in both males and females. Reasons for the decline in GSI with increasing fish size probably relate to the nest guarding nature of this species. Unlike the case of broadcast spawners who invest nothing in parental care and simply attempt to produce as many gametes as possible, fish that invest in parental care have a finite capacity to guard and care for the egg mass meaning there is no advantage to a female producing more eggs than a single male is capable of caring for (unless she lays in more than one nest which is a possibility but yet to be tested experimentally) and no advantage for a male in producing more sperm than is necessary to fertilise those eggs.

4.3 Seminal Vesicle Somatic Index

SVSI showed a distinctive seasonal trend with seminal vesicle investment peaking during spring and beginning to decline over summer. Investment in seminal vesicles dropped to minimum investment over autumn and winter months especially April and May. During this time, the production of seminal fluid is thought diminish over the period of reproductive dormancy was described in a study by Nayyar & Sundararaj (1970) on the catfish, *Heteropneustes fossilis.* The low SVSI over this period is thought to be the empty or basal weight of the organ. In general male SVSI showed a similar seasonal trend in terms of investment to female GSI although GSI as a percentage of body weight in females was far higher than male SVSI. This portrays the high seasonal variation in male SVSI and female GSI compared to the relatively gradual seasonal change in male GSI.

Male SVSI/GSI ratio show distinct peaks in summer and spring. This suggests that the high seasonal variability of the seminal vesicle mass is

controlling the SVSI/GSI ratio with seasonal changes in energetic investment in gonads having little effect on this ratio. When plotted against total fish length, male SVSI/GSI ratio showed a positive correlation. This data is limited by catch based selectivity as fish with an estimated total length less than 40 mm were put back as they were most likely not reproductively active. There were also very few males that were caught that were larger than 70 mm in total length. This may be attributed to the population dynamics of this particular lacustrine habitat rather than selectivity in the sampling methodology. Total length of bully populations residing in lacustrine habitats are thought to be smaller on average than bully populations residing in riverine habitats (Bleackley, 2008). There were very few larger individuals (65mm+) but they did not display an exceeding large SVSI/GSI ratio as one might expect. Santos et al (1996) suggests that this may be due to male reproductive organs having an optimal size above which no reproductive benefits can be gained by increasing seminal fluid or sperm production.

The tendency for larger males to develop relatively larger seminal vesicles strongly suggests that these individuals are more likely to establish and guard nest sites. This can be seen in a study by Immler et al (2004) which showed increased seminal vesicle size with ‘parental’ males in the black goby, *Gobius niger* (Immler et al, 2004).

4.4 Somatic condition

Female somatic condition trends appear to be relatively stable throughout the 14 month sampling period with slight increases over the spring and summer periods. December 2013 and January 2015 show spikes in female somatic condition variability which can be attributed to a decrease in the number of females in the catch throughout those months. It is likely that this variability would stabilise with a consistent catch sex ratio. Female condition was most stable throughout autumn and winter with very little variability overall.

Male somatic condition trends also appear to display a stable mean throughout autumn and winter with only slightly increased variability compared to female somatic condition during these months. Summer 2013/2014 showed a noticeable increase in both mean somatic condition as well as variability however, our overall catch numbers were smallest during these three months. The 2014/2015 summer data showed the same slight increase in trend and variability however it is more tightly grouped which may be attributed to the increase in catch number.

When compared to seasonal condition of common bully in Lake Waahi (Stephens et al, 1978), Stephens found that somatic condition was not only most stable, but also highest throughout winter. This was attributed to increased investment in somatic growth outside of the spawning season. The trend of increased somatic condition throughout winter was not reflected in this study however, the stability in condition was. It is possible that the increased trend in somatic condition over the summer period as seen in Figure 3.10 can be linked to an increase in food availability in the lake during this time.

The increased variability displayed in the male somatic condition index may also be affected by the acts of brood care and nest defence occurring throughout the spring and summer spawning periods for a percentage of males in the population. A condition based study by Skolbekken & Utne-Palm (2001) focused on the comparative somatic condition of nest guarding and non-nest guarding male two-spotted goby (*Gobiusculus flavescens*) during the spawning season. The authors found that somatic condition decreases with nest guarding males but no obvious decrease was seen for non-nest guarding males during that time frame. This suggests that the physical act of guarding and brood care has a noticeable negative affect on the somatic condition of the nest guarding males (Skolbekken & Utne-Palm, 2001).

4.5 Catch sex ratio

Throughout the 14 month sampling period, female catch sex ratio never went over 50% of the total catch, depicting a male dominant population. Male catch ratio was exceedingly high (peaking at 90%) throughout summer and began to decline during autumn and winter. Male catch was low and most stable throughout late winter and spring. This is most likely due to nest guarding males receding from the sampling sites during this period to begin cleaning and guarding a nesting territory in preparation for the spawning season which first peaks in August (Stephens, 1978). Alternatively, the increase in male catch throughout the late spawning period may be the result of increased activity of non-nest guarding males actively attempting to seek out nests to "target" using non-bourgeois reproductive techniques.

4.6 Behavioural Experiment

In early December 2014 we began the week long behavioural experiment designed to investigate the nest site selection of both male and female bullies as well as the mate selection by females. Four different treatments were designed to examine possible influences in mate choice and male reproductive behaviour, such as male size and competition.

The treatments were chosen to investigate multiple interactions with different numbers of males and females to compare male size and competition for optimal nest sites. Multiple females were used to investigate the influencing factors of mate selection and determine if one factor appears to be most important or if it is a combination of factors. The two main factors that we focused on were male size and nest size which were also the focus of a study by Hamilton et al (1997) on *G. breviceps* in two Otago streams. The results of the Hamilton et al. study showed no significant correlation between male reproductive success and the two traits, although the authors suggest that this may not be a true representation of the population. Previous studies acknowledge the importance of male size and nest size in female mate choice in the mottled sculpins *(Cottus bairdi*) and the threespine stickleback,

(*Gasterosteus aculeatus)* (Downhower & Brown, 1980, Sargent, 1982, Sargent & Gebler, 1980) but due to small sample sizes and high variability in the experimental habitat, this was not observed in the study by Hamilton et al (1997).

Unfortunately, we were faced with an unforeseen difficulty throughout the course of the experiment regarding fish health and subsequent death. On day two of the experiment fish began dying which continued consistently throughout the course of the week. We decided to finish the experiment as fish that survived were showing signs of nest guarding and spawning but data gathered was incomplete due to sickness and death of the fish. At the end of the week experimental conditions were thoroughly cleaned and dried in preparation for a second attempt. Two days before the second attempt, we collected the subjects and brought them into the lab for sexing in preparation for the experiment. However within 24 hours approximately 25% of the fish were showing obvious symptoms of fin rot and white spot, and within 48 hours these fish were dead and most of the remaining fish displayed symptoms resulting in death. Within four days, all of the fish brought into the lab had died. This pattern was replicated to varying degrees with two more separate lots of fish over a period of a month as treatment only seemed to delay the inevitable for 24-48 hours. Due to time constraints of the research project and seasonality of spawning, further attempts of the experiment were aborted.

Numerous preventative measures had been used to help reduce fish stress and prevent fungal and bacterial infections as well as treatments after symptoms presented. This included: set lab water temperature of 15˚C in addition to artificial seawater added to the tank at a ratio of 1:10 to help reduce stress as well as fungal and bacterial infections. To help reduce bacterial and nutrient build up, a 1/3 water change occurred daily and fish were fed sparingly three times a week. We added a product called STRESS COAT ® (API) to the water that the fish were transported in as well as the water they were housed in. STRESS COAT® is an aloe vera based product designed to replenish and buffer the protective slime

coating on the fish scales which helps to reduce the penetrative ability of bacterial and fungal infections.

When symptoms presented, the fish were medicated with malachite green in formalin to treat *Ichthyophthirius* (white spot). We found that a number of fish were already contaminated with white spot in the field, so we did not bring these fish into the lab, and fish that presented with symptoms were removed from the main tanks and treated separately. If it was apparent that the fish were suffering or past the point of treatment, then they were humanely euthanized in accordance with University of Waikato standard operating procedure 6, Euthanasia and Anaesthesia of Fish (Ling, 2006a).

This experiment has a lot of potential for future studies, with a few considerations and minor adjustments. The first and most important of which is the time of the season that the experiment runs. There was very little data to consult when choosing the optimal week for the experiment to run as the spawning timing of common bully is often influenced by habitat type and had not been established for this population. Timing was therefore based on data taken from Stephens (1978) which showed there to be three main peaks in the spawning activity in the population from Lake Waahi (shallow Waikato lake) this was during August, November and February. Animal Ethics permission was not granted until August and construction of the pots took a number of weeks, February was too close to the commencement of this thesis so late November was decided on but then postponed to early December due to adverse weather conditions for fishing.

The week of the experiment, I recorded the daily ambient air temperatures as well as the water temperatures of the outside tanks where the experiment took place. The average air temperature for the week was 26˚C and the average water temperature for the week (taken daily at 3pm) was 24.3˚C, this is 4.1˚C higher than their preferred temperature of 20.2˚C as noted by Richardson et al (1994). Stress is known to adversely affect freshwater fish, increasing chances of disease and infection (Peters, 1988)

and can be triggered by changes in temperature, handling and water quality (Richardson et al, 1994). “While fish can survive, within limits, in temperatures outside their optimal ranges, resulting physiological or behavioural changes can decrease their chances of survival and reproductive success” (Reynolds 1977). After consultation with a number of fish biologists at the University of Waikato and local fish care experts, thermal stress was considered to be the common factor initiating the susceptibility to white spot and fungal infections whilst still in their natural habitat as this was not seen throughout the rest of my monthly sampling when the seasonal temperatures were lower. The additional stress of field collection, handling and water chemistry changes was thought to amplify the spread of the infection in captivity, causing the rapid decline in fish health resulting in death. We had a few cases of white spot during the summer sampling of 2013/2014 but as catch numbers were lower, the fish were dissected within 24 hours therefore symptoms did not have time to develop in holding tanks.

Although the majority of the data gained throughout the course of the week long experiment was unsuitable for statistical analysis, we were able to gain insights from the data regarding male nest preference. This data shows that the large nest pot was the most favourable choice out of the large and small options, with the small pot often only being selected as a nesting site if the large pot was already occupied. GSI and SVSI data from the males within the experiment suggest an increased investment in seminal vesicles by males displaying nest guarding behaviour. However, this sample size for both of these elements was too small to provide statistically significant results.

4.7 General Summary

Throughout the course of this research it was found that investment in gonads and seminal vesicles (in males) increase in preparation for the breeding season. However the increase in SVSI was noticeably more drastic than the comparatively slight increase in GSI which shows less variability throughout the seasons. Female GSI calculations showed

similar trends to that of the male SVSI with sharp increases occurring throughout late winter and spring. This suggests energetic investment in reproduction is focused solely on gonad development in females, but is split between gonads, seminal vesicles, brood care and nest guarding in males. Reproductive investment in male gonads was observed (during dissections) as being variable with some males investing heavily in gonads compared to seminal vesicles however, due to the nature of the seasonal data this is not easily observable in the results as they often appear as outliers. This variation in seminal vesicle and gonad size was expected based on our hypothesis of differential investment in reproductive structures as an indicator of MART's. In general, male gonad investment was relatively stable with gradual increases during the spawning season. This can be compared with the seasonally limited investment in seminal vesicles that showed evident peaks and declines with changing seasons.

A behaviour based experiment was designed and run to investigate mate preferences, nest site selection and male nest guarding behaviours. The limited results we gained from running this experiment showed an obvious preference in nest site selection of the larger of the two nest pot choices available. Dissections of the remaining fish from the behavioural experiment showed that fish displaying nest guarding behaviour invested more heavily in seminal vesicles than non-nest guarding fish. There was also little difference in average comparative investment of GSI and SVSI in non-nest guarding males and increased investment in SVSI compared to GSI in nest guarding males.

The SVSI/GSI ratio of the dissected males showed a significant difference between nest guarding and non-nest guarding males providing promising evidence in support of the possibility of MART's in the *Gobiomorphus*. The nest site selection aspect of the experiment showed an obvious preference for larger nesting pots over smaller nest pots and where male size was compared, the larger of the two males often chose the large pot, with the small male guarding the small pot or not displaying guarding behaviour at all.

The general conclusions that can be draw from this research are:

- There is a strong positive correlation between reproductive investment in both gonads and seminal vesicles with spawning seasonality.
- Investment in SVSI and GSI can differ considerably between individuals.
- Males showed preference of large nesting sites over small nesting sites.
- Males displaying nest guarding behaviour on average had an increased investment in seminal vesicles than non-nest guarding males.
- A portion of males showed a considerably larger investment in gonads than other males, supporting the possibility of multiple alternative reproductive techniques in *Gobiomorphus cotidianus.*

4.8 Future Research

There is a huge potential for future studies stemming from this research, including a more comprehensive investigation into the comparative reproductive investment of both male and female common bully not only in physiological investment but including calculations of energetic investment in male brood care and nest defence of guarding males.

A more in-depth study identifying paternity of broods via genetically testing the offspring would show the effectiveness of brood care and nest defence compared with other reproductive techniques. This would allow identification of the paternal father of the offspring which could then be cross referenced against the DNA of the nest guarding male. This would assess the percentage of success nest guarding behaviour had as opposed to other reproductive techniques by providing a ratio of paternal input.

Due to the compromised health of the population of fish used for our behavioural experiment, we were unable to gain statistically significant

data with an appropriate amount of treatment replicates. However, with lessons learnt from this study including timing and optimal fish health, I believe that the results gained from a further attempt at this experiment would fill numerous gaps in adding to the knowledge base surrounding the reproductive biology of this species. Variations of this experiment could also include placing artificial nest sites into an established population in a lake or river and monitoring it each day by scuba to evaluate nest choice and guarding whilst eliminating limitations imposed by laboratory conditions.

With more time and funding I would have liked to include comparisons between the population in Lake Karapiro and another established population in a riverine habitat to provide a spatial aspect to the study. I would have also liked to include different species within the New Zealand Gobiomorphus to identify any changes in trends between species of the same genus, but the time and funding restraints of a Master's degree prevented this venture.

Physiological evidence was found to support the possibility of MART's occurring in this species. Further research should be undertaken to investigate (on a larger scale) the reproductive behaviour of this family under both controlled laboratory conditions as well as in their natural habitats to definitively confirm or deny the presences of MART's within the Gobiomorphus.

References

Berra, T. M. (2001). Freshwater fish distribution. *Academic Press.*

Bleackley, N. A. (2008). Biology of common bully *(Gobiomorphus cotidianus)* populations in the Tarawera and Rangitaiki rivers: Reproductive isolation by inland distance or effluent discharges?

Bleackley, N. A., Landman, M. J., & Ling, N. (2009). Ecology of common bully (*Gobiomorphus cotidianus*) in the Tarawera and Rangitaiki rivers: Isolation by inland distance or anthropogenic discharge? *New Zealand Journal of Marine and Freshwater Research, 43(4), 889-899.*

Cadwallader, P. L. (1975). Feeding relationships of galaxiids, bullies, eels and trout in a New Zealand river. *Australian Journal of Marine and Freshwater Research 26, 299–316.*

Cargnelli, L. M., & Gross, M. R. (1996). The temporal dimension in fish recruitment: birth date, body size, and size-dependent survival in a sunfish (bluegill: *Lepomis macrochirus*). *Canadian Journal of Fisheries and Aquatic Sciences*, *53*(2), 360-367.

Cinquetti, R., & Rinaldi, L. (1987). Changes in the gonadal histology of *Padogobius martensi (Pisces: Gobiidae)* during the reproductive cycle. *Italian Journal of Zoology, 54(3), 233-241.*

Closs, G.P., Smith, M., Barry, B., Markwitz, A. (2003). Non-diadromous recruitment in coastal populations of common bully (*Gobiomorphus cotidianus*). *New Zealand Journal of Marine and Freshwater Research, 37: 301-313.*

Downhower, J.E & Brown, L. (1980). Mate preferences of female mottled sculpins *Cottus bairdi. Animal Behaviour 28: 728-734.*

Duarte, C. M., & Alcaraz, M. (1989). To produce many small or few large eggs: a size-independent reproductive tactic of fish. *Oecologia, 80(3), 401-404.*

Dunbar, R. I. M. (1982). Intraspecific variations in mating strategy. In *Ontogeny* (pp. 385-431). Springer US.

Gomon, M. F. (2011a). Striped Gudgeon, *Gobiomorphus australis*, in Fishes of Australia, accessed 03 February 2015, http://www.fishesofaustralia.net.au/home/species/4142

Gomon, M. F. (2011b). Cox's Gudgeon, *Gobiomorphus coxii,* in Fishes of Australia, accessed 03 February 2015, http://www.fishesofaustralia.net.au/home/species/4143

Goodman, J. M., Dunn, N. R., Ravenscroft, P. J., Allibone, R. M., Boubee, J. A. T., David, B. O., Griffiths, M., Ling, N., Hitchmough, R. A. and Rolfe, J. R. (2013). Conservation status of New Zealand freshwater fish, Department of Conservation, New Zealand.

Gross, M. R. & Sargent, R. C. (1985). The evolution of male and female parental care in fishes. *American Zoologist 25, 807–822.*

Gross, M. R. (1982). Sneakers, satellites and parentals: polymorphic mating strategies in North American sunfishes. *Z. Tierpsychol. 60:1-26.*

Gross, M. R. (1984). Sunfish, salmon, and the evolution of alternative reproductive strategies and tactics in fishes. In: Fish Reproduction: Strategies and Tactics (Ed. by G. W. Potts & R. J. Wootton), *pp. 55-75. London: Academic Press.*

Hamilton, W. J. & Poulin, R. (1999). Female preference and male nuptial colouration in the freshwater fish *Gobiomorphus breviceps*: geographic variation among populations. *Canadian journal of zoology, 1999 - NRC Research Press.*

Hamilton, W. J. (1998). Threat-sensitive Nest Preferences in Male Upland Bullies, *Gobiomorphus breviceps. Ethology, 104(8), 709-715.*

Hamilton, W. J., Stott, M. K., & Poulin, R. (1997). Nest site characteristics and male reproductive success in the upland bully, *Gobiomorphus breviceps (Eleotridae). Ecology of Freshwater Fish, 6(3), 150-154.*

http://nzwaterways.co.nz/case-studies/ CASE STUDY | 1995 Lake Eland (Hawke's Bay) retrieved (28th January 2015)

Immler, S., Mazzoldi, C., & Rasotto, M. B. (2004). From sneaker to parental male: change of reproductive traits in the black goby, *Gobius niger (Teleostei, Gobiidae*). *Journal of Experimental Zoology Part A: Comparative Experimental Biology, 301(2), 177-185.*

Jonsson, N., Jonsson, B., & Hansen, L. P. (1991). Energetic cost of spawning in male and female Atlantic salmon *(Salmo salar L.). Journal of Fish Biology, 39(5), 739-744.*

Kodric-Brown, A., & Brown, J. H. (1984). Truth in advertising: the kinds of traits favored by sexual selection. *American Naturalist, 309-323.*

Kolm, N., & Ahnesjö, I. (2005). Do egg size and parental care coevolve in fishes? *Journal of Fish Biology, 66(6), 1499-1515*.

Lahnsteiner, F., Seiwald, M., Patzner, R. A., & Ferrero, E. A. (1992). The seminal vesicles of the male grass goby, *Zosterisessor ophiocephalus (Teleostei, Gobiidae). Zoomorphology, 111(4), 239-248.*

Landman, M. J., Bleackley, N. A., Ling, N., & Heuvel, M. R. (2010). Physiological health of common bully (*Gobiomorphus cotidianus*) in the Tarawera and Rangitaiki Rivers of New Zealand: evidence of diminished ecological effects of pulp and paper effluents in wild fish populations. *Water Quality Research Journal of Canada, 45(2), 223–233.*

Lehtonen, T. K., & Lindström, K. (2008). Repeatability of mating preferences in the sand goby. *Animal Behaviour, 75(1), 55-61.*

Lindström, K. & Hellström, M. (1993) Male size and parental care in the sand goby, *Pomatoschistus minutus*. *Ethology Ecology Evoloution, 5: 97–106*

Ling, N. (2006)a. University of Waikato Animal Ethics Committee, standard operating procedure, SOP Number: 6, Euthanasia and anaesthesia of fish.

Ling, N. (2006)b. University of Waikato Animal Ethics Committee, standard operating procedure, SOP Number: 7, Capture, handling and captive maintenance of fish.

Locatello, L., Pilastro, A., Deana, R., Zarpellon, A., & Rasotto, M. B. (2007). Variation pattern of sperm quality traits in two gobies with alternative mating tactics. *Functional ecology, 21(5), 975-981.*

Magnhagen, C. (1992). Alternative reproductive behaviour in the common goby, *Pomatoschistus microps*: an ontogenetic gradient. *Animal Behaviour, 44, 182-184.*

Magnhagen, C. (1994). Sneak or challenge: alternative spawning tactics in non-territorial male common gobies. *Animal Behaviour, 47, 1212-1215.*

Magnhagen, C. (1998). Alternative reproductive tactics and courtship in the common goby. *Journal of Fish Biology, 53(1), 130-137*.

Malavasi, S., Torricelli, P., Lugli, M., Pranovi, F., & Mainardi, D. (2003). Male courtship sounds in a teleost with alternative reproductive tactics, the grass goby, *Zosterisessor ophiocephalus. Environmental biology of fishes, 66(3), 231-236.*

Manica, A. (2002). Filial cannibalism in teleost fish. *Biological Reviews of the Cambridge Philosophical Society, 77(02), 261-277.*

Marconato, A., Rasotto, M. B., & Mazzoldi, C. (1996). On the mechanism of sperm release in three gobiid fishes *(Teleostei: Gobiidae)*. *Environmental Biology of Fishes, 46(3), 321-327.*

Marentette, J. R., Fitzpatrick, J. L., Berger, R. G., & Balshine, S. (2009). Multiple male reproductive morphs in the invasive round goby *(Apollonia melanostoma). Journal of Great Lakes Research, 35(2), 302-308.*

Mazzoldi, C., Petersen, C. W., & Rasotto, M. B. (2005). The influence of mating system on seminal vesicle variability among gobies (*Teleostei, Gobiidae*). *Journal of Zoological Systematics and Evolutionary Research, 43(4), 307-314.*

McDowall, R. M. (1990). New Zealand Freshwater Fishes: A Natural History and Guide. *Heinemann Reed, Auckland.*

McDowall, R. M. (1993). Implications of diadromy for the structuring and modelling of riverine fish communities in New Zealand. *New Zealand Journal of Marine and Freshwater Research, 27: 453–462.*

McDowall, R. M. (1997). The evolution of diadromy in fishes (revisited) and its place in phylogenetic analysis. *Reviews in Fish Biology and Fisheries, 7(4), 443-462.*

McDowall, R.M. (1975). A revision of the New Zealand species *Gobiomorphus (Pisces: Eleotridae)*. *National History Museum of NZ records, 1(1):1-32.*

McDowall, R.M. (1994). The Tarndale bully, Gobiomorphus alpinus Stokell (*Pisces: Eleotridae*) revisited and redescribed*. Journal of the Royal Society of New Zealand, 24: 117-124.*

McDowall, R.M. (2001). Diadromy, diversity and divergence: implications for speciation processes in fishes. *Fish and* Fisheries*, 2: 278 - 285.*

McDowall, R.M., Stevens, M.I. (2007). Taxonomic status of the Tarndale bully *Gobiomorphus alpinus* (*Teleostei: Eleotridae*), revisited—again*. Journal of the Royal Society of New Zealand, 37: 15–29.*

Michel, C., Hicks, B. J., Stölting, K. N., Clarke, A. C., Stevens, M. I., Tana, R., ... & Van den Heuvel, M. R. (2008). Distinct migratory and non-migratory ecotypes of an endemic New Zealand eleotrid (*Gobiomorphus cotidianus*) – implications for incipient speciation in island freshwater fish species. *BMC Evolutionary Biology, 8(1), 49.*

Nagahama, Y. (1983). The functional morphology of teleost gonads. *Fish physiology, 9(Part A), 223-275.*

Nayyar, S. K. & Sundararaj, B. I. (1970). Seasonal reproductive activity in the testes and seminal vesicles of the catfish, *Heteropneustes fossilis (Bloch). Journal of Morphology, 130(2), 207-225.*

NIWA Freshwater Fish Database, common bully known distribution. Retrieved (12th January 2015) https://www.niwa.co.nz/freshwater-and-estuaries/nzffd/NIWA-fish-atlas/fish-species/common_bully

NIWA Freshwater Fish Database, common bully penetration. Retrieved (12th January 2015) https://www.niwa.co.nz/freshwater-and-estuaries/nzffd/NIWA-fish-atlas/fish-species/common_bully

Northcote, T.G. & Ward, F.J. (1985). Lake resident and migratory smelt *Retropinna retropinna* (Richardson), of the lower Waikato River system. *New Zealand Journal of Marine and Freshwater Research 17, 113–129.*

Okuda, N., Ito, S. & Iwao, H. (2003). Female mimicry in a freshwater goby *Rhinogobius sp. OR*, *Ichthyology Research 50: 198–200.*

Oliveira, R. F., Taborsky, M., & Brockmann, H. J. (Eds.). (2008). Alternative reproductive tactics: *an integrative approach*. Cambridge University Press.

Perrone Jr, M., & Zaret, T. M. (1979). Parental care patterns of fishes. *American Naturalist, 351-361.*

Peters, G., Faisal, M., Lang, T. & Ahmed, I. (1988) stress caused by social interaction and its effects on susceptibility to *Aeromonas hydrophilia* infection in rainbow trout *salmo gairdneri. Diseases of aquatic organisms, 4: 83-89.*

Poulin, R. (1994). Mate choice decisions by parasitized female upland bullies, *Gobiomorphus breviceps*. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 256(1346), 183-187.*

Rasotto, M. B., & Mazzoldi, C. (2002). Male traits associated with alternative reproductive tactics in *Gobius niger. Journal of fish biology, 61(1), 173-184.*

Reynolds, W. W. (1977).Temperature as a proximate factor in orientation behaviour. *Journal of the Fisheries Research Board of Canada 34: 734-739.*

Richardson, J., Boubée, J. A., & West, D. W. (1994). Thermal tolerance and preference of some native New Zealand freshwater fish. *New Zealand journal of marine and freshwater research, 28(4), 399-407.*

Santos, R. S., Hawkins, S. J., & Nash, R. D. M. (1996). Reproductive phenology of the Azorean rock pool blenny a fish with alternative mating tactics. Journal of fish biology, 48(5), 842-858.

Sargent, R. C. & Gross, M. R. (1986). Williams' principle: an explanation of parental care in teleost fishes. *In The Behaviour of Teleost Fishes (Pitcher, T. J., ed.), pp. 275–293.*

Sargent, R. C., Taylor, P. D., & Gross, M. R. (1987). Parental care and the evolution of egg size in fishes. *American Naturalist, 32-46.*

Sargent, R.C & Gebler, J.B. (1980). Effects of nest site concealment on hatching success, reproductive success, and parental behaviour of the threespine stickleback, *Gasterosteus aculeatus. Behavioural Ecology and Sociobiology 7: 137-142.*

Sargent, R.C. (1982). Territory quality, male quality, courtship intrusion and female nest-choice in the threespine stickleback, *Gasterosteus aculeatus. Animal Behaviour 30: 364-374.*

Skolbekken, R., & Utne-Palm, A. C. (2001). Parental investment of male two-spotted goby, *Gobiusculus flavescens (Fabricius). Journal of experimental marine biology and ecology, 261(2), 137-157.*

Smith, P. J., McVeagh, S. M., & Allibone, R. (2005). Extensive genetic differentiation in *Gobiomorphus breviceps* from New Zealand. *Journal of Fish Biology, 67(3), 627-639.*

Smith, P.J., McVeagh, S.M., Allibone, R. (2003). The Tarndale bully revisited with molecular markers: an ecophenotype of the common bully *Gobiomorphus cotidianus (Pisces: Gobiidae). Journal of the Royal Society of New Zealand, 33: 663-673.*

Stephens, T. (1978). The Biology of *Gobiomorphus Cotidianus (Pisces: Eleotridae)* in Lake Waahi (Doctoral dissertation, University of Waikato).

Stephens, R.T.T. (1982). Reproduction, growth and mortality of the common bully, *Gobiomorphus cotidianus* McDowall, in a eutrophic New Zealand lake. *Journal of Fish Biology, 20: 259-270.*

Stoltz, J. A., Neff, B. D., & Olden, J. D. (2005). Allometric growth and sperm competition in fishes. *Journal of fish biology, 67(2), 470-480.*

Stott, M.K., & Poulin, R. (1996). Parasites and parental care in male upland bullies (*Eleotridae*) *Journal of Fish Biology 48, 283–291.*

Sutherland, D. L., & Closs, G. P. (2001). Spatial and temporal variation in the abundance and composition of ichthyoplankton in a large South Island, New Zealand river estuary*. New Zealand Journal of Marine and Freshwater Research, 35(5), 1061-1069.*

Taborsky, M. (1994). Sneakers, satellites, and helpers: parasitic and cooperative behaviour in fish reproduction. *Advanced Study Behaviour, 23, l-100.*

Taborsky, M. (1997). Bourgeois and parasitic tactics: do we need collective functional terms for alternative reproductive tactics? *Behavioural Ecology and Sociobiology, 41:361-362.*

Taborsky, M. (1998). Sperm competition in fish: Bourgeois males and parasitic spawning. *Trends in Ecology and Evolution, 13:222-227.*

Taborsky, M. (2001). The evolution of bourgeois, parasitic, and cooperative reproductive behaviors in fishes. *Journal of Heredity, 92(2), 100-110.*

Taborsky, M. (2008). 10· Alternative reproductive tactics in fish. *Alternative Reproductive Tactics An Integrative Approach*, 251.

van den Heuvel, M.R., Michel, C., Stevens, M.I., Clarke, A.C., Stölting, K.N., Hicks, B.J., Tremblay, L.A. (2007). Monitoring the effects of pulp and paper effluent is restricted in genetically distinct populations of common bully (*Gobiomorphus cotidianus*). *Environmental Science & Technology, 41: 2602- 2608.*

Vanderpham, J. P. (2012). Habitat-related variation of the lateral-line mechanosensory system of the habitat-specialist redfin bully

and habitat-generalist common bully (Thesis, Doctor of Philosophy). University of Otago.

Vanderpham, J. P., Nakagawa, S., & Closs, G. P. (2013). Habitat-related patterns in phenotypic variation in a New Zealand freshwater generalist fish, and comparisons with a closely related specialist. *Freshwater Biology, 58(2), 396-408.*

Withers, L. (1950). The development of hydroelectric power in New Zealand. *New Zealand Geographer, 6(1), 53-65*.